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REPORT OF ACTIVITIES OF A. A. C. C. BAKING RESEARCH FELLOWSHIP [1]

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This is the final report of the laboratory activities of the first A. A. C. C. Baking Research Fellowship project. The experimental work herein recorded is primarily that which has been done subsequent to the publication of the previous report (Merritt and Blish, 1931). In addition to a statement of actual laboratory results, this report contains general and specific conclusions, recommendations, and suggestions by the Research Fellow and his associates, based upon a survey and consideration of observations and findings throughout the entire period of the Fellowship.

The immediate outcome of the Research Fellow's activities will doubtless disappoint any individual who may have hoped that the results would permit a final establishment of all specifications, both for performance and interpretation of the test, that will adequately serve all purposes and requirements pertaining to the ascertainment of flour baking characteristics. Many years will elapse before this ideal situation in experimental test baking can be approached to the same degree and in the same sense that obtains with most of the other types of chemical and physical testing procedures with which the cereal technologist is familiar. As was clearly shown by the work of Herman and Hart (1927), the critical factors encountered in the baking test far out-number those involved in, for example, the ash, moisture, and protein tests. Yet even these simpler tests cannot be considered to have, as yet, reached the ideal state. They were in a condition of continuous evolution, modification, and improvement many years before any standardized baking test was ever attempted, and they have not, even yet, passed beyond that stage.

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First of all, it is perhaps appropriate that the Research Fellow and his associates state in very general terms what they think has been accomplished by the project as a whole, and there should also be a statement indicating the general nature of factors that have imposed definite limitations upon the scope of the Fellow's activities.

It is believed by the writers that the principal items of accomplishment are: (1) An improved knowledge and appreciation of the relative importance of some of the more conspicuous factors that affect both the baking test and its interpretation; (2) the securing of information as to the probable error of individual tests, and as to factors affecting variability; (3) modifications of procedure for special purposes and requirements; (4) the gaining of knowledge regarding types of equipment designed both for the convenience of the operator and for greater elimination of the personal element in manipulation; and, (5) suggestions of appropriate subjects for future investigations.

Perhaps the most serious handicap throughout the work has been the impossibility of having adequate contacts and consultations with members of the Standard Baking Test Committee and others whose advice, suggestions, and criticisms at frequent intervals would no doubt have led to better planning and organization of the laboratory work. Every effort has been made to deal with items of general rather than special interest. It is probably true, however, that certain phases of the work have been over-emphasized while others failed to receive the attention they deserve.

Due to the wide diversification of interests among the members of the A. A. C. C., as well as of other contributors to the Fellowship fund, it has been deemed advisable to cover a fairly large range of territory and to deal with a variety of topics. The disadvantages of this type of procedure are obvious, but it is felt that under the circumstances the disadvantages are outweighed by the advantages of such a system of study. Although this method of operation does not permit of the deep and thorough cultivation of any one field, it is believed that in the majority of instances, enough work has been done on each topic to show definite trends. To have entirely exhausted the possibilities in any single phase of the work would have necessitated the complete neglect of other important issues, and the program would then have been all the more open to charges of misplaced emphasis.

In an endeavor to reduce variability, eliminate the personal element, avoid the necessity for requiring elaborate and excessively expensive equipment, while at the same time keeping the test as informative as possible, many difficulties and complications were encountered. In considering any single modification of procedure, conflicting viewpoints invariably arise. The nature of these can be

best illustrated by citing an individual case. One phase of the work has involved the possible substitution of hand punching and hand molding by the use of a single set of sheeting rolls. This is convenient, and it should reduce variability by greatly minimizing the personal element in handling. On the other hand, many operators maintain that they like to get the *feel* of the dough during punching and molding, and that they secure much valuable information thereby. Furthermore, the rolls constitute an extra item of equipment, although they would be far less expensive than any mechanical molder now on the market. Another noticeable feature of the rolls is that in giving a closer and more uniform crumb structure they tend to minimize variations in crumb characteristics, and no doubt some will object to this, claiming that it makes the test less informative. But this, in turn raises the question as to what significance should be attached to minor variations in grain and cell structure anyway, if these are so profoundly influenced merely by the method of molding. Doubtless much of the significance that is placed upon some of these items in bread scoring is fictitious rather than real.

The foregoing illustrates a type of situation that has frequently presented itself during the course of the work. The rendering of final judgement in matters of this character involves the balancing of several divergent viewpoints against each other, and is no simple matter. There are certain issues where it seems unsafe to attempt a final decision until experiments have been made with a far greater range of flour types than could be handled under the conditions of the Fellowship.

Another type of complication was encountered in studying the comparative responses of different flours to almost any *differential* treatment involving a single factor applied in varying amounts, or in different ways, or to different degrees. Thus, for example, one flour may be sensitive to different types of molding treatment, while with another flour the method of molding is apparently of no critical importance. With one flour the volume differential when baked in two different types of baking pans may be large, while with another flour it is insignificant. These and similar situations emphasize the necessity of being extremely cautious in recommending the alteration of any of the specifications of the present basic procedure (Blish, 1928).

To adequately record and report all phases of the Research Fellow's experiences would be as impossible as it would be useless. There were occasional inconsistencies for which no explanations can be offered. There were a few brief excursions into blind alleys. A few small portions of data that proved to be of no consequence or significance have purposely been omitted from the report as their presentation

would serve no useful purpose. However, more than 90% of the data are regarded as significant and are summarized here.

Comments on tabular material, figures, etc., are purposely and necessarily brief, since, in most cases, the evidence which they present and the conclusions to be drawn from them, are obvious enough to those who are sufficiently interested to examine the data closely.

Attempts to describe internal loaf characteristics are omitted where this item has no important bearing upon the main issue. The reader's familiarity with the details of the present tentative standard A. A. C. C. basic procedure is assumed throughout the report. Some familiarity with the previous report (1931) is also assumed.

Experimental

Additional Mixing Studies

The previous report of the Research Fellow (1931) favored the Hobart-Swanson mixer as now designed for use with doughs from 200 gms. of flour. A mixing time of 1 minute was recommended for the basic procedure, and it was shown that increased loaf volume could ordinarily be expected where the mixing time was increased to 2 or 3 minutes. The further mixing studies to be recorded here involve the use of the usual Hobart mixer equipped with 2 dough hooks. This type of mixing is frequently used in laboratory baking tests, and has been habitually used in the extensive work of the Associate Committee on Grain Research of the National Research Council of Canada, as indicated by Larmour, Machon and Brockington (1931).

A few preliminary trials indicated that 3 to 4 minutes of mixing at intermediate speed, using the 2 dough hooks, gave results closely approximating those secured by a 1-minute-mix with the new Hobart-Swanson mixer.

TABLE I

Mixing Studies with Two Dough Hooks[1]

Date	Mixing time	Number of individuals	Mean volume	Standard deviation	Coefficient of variability
	Min.		cc.		
			Flour No. 3		
5/28/31	4	30	592	9.77	1.31
6/1	4	30	574	6.13	1.07
6/2	4	30	591	8.28	1.40
			Flour No. 4		
5/26	4	30	605	13.49	2.23
5/27	4	30	605	10.36	1.71
5/29	4	30	582	9.79	1.68

[1] Doughs from 200 gms. flour divided into two equal portions after mix.

Table I gives loaf volumes and variability coefficients resulting from the use of the Hobart bowl with the 2 dough hooks. The baking was done in oven *Y*, and all the doughs were machine molded.

A comparison of the data in Table I with similar data in the previous report (1931) shows that this type of mixing gave loaf volumes and variabilities of the same order of magnitude as were obtained by a 1-minute-mix with the Hobart-Swanson mixer. The Hobart-Swanson mixer saves time and is easier to clean. A further disadvantage of the 2 hooks is that the adjustment of their shape, conformity to the shape of the bowl, and clearance from the bottom of the bowl, are critical factors that are difficult to standardize.

Another matter for consideration is the suitability of the 2 hooks for studies involving supplementary method D, which deals with tolerance to prolonged mixing, or resistance to mechanical modification. For this purpose the Hobart-Swanson mixer is decidedly preferable. This device gave thorough and uniform mixing under all conditions, and *modified* the gluten in a much shorter time than did the 2 dough hooks. On the other hand, although the 2 hooks gave a fairly uniform mixing during 3 or 4 minutes at intermediate speed, their behavior became erratic when mixing at high speed and for extended time intervals. As the gluten became *developed* there was a tendency for the dough to *climb* the hooks. If the dough became slightly overdeveloped and sticky, there was a marked tendency for a portion of it to remain in the bottom of the bowl and fail to be picked up by the hooks.

Considering all angles of the mixing studies that have been undertaken by the Research Fellow, there is justification for giving a definite and decided preference to the Hobart-Swanson mixer in its present form. From the standpoint of thorough and uniform mechanical mixing, in the shortest time, and at a maximum of convenience to the operator, it is superior to all other types of mechanical mixing that have been dealt with in these studies. It must be remembered, however, that in its present form it requires 200 gms. of flour for best results. This is no serious disadvantage, however, as the dough can be easily divided into 2 equal portions, and one generally bakes in duplicate anyhow.

Punching and Molding Studies

Hand manipulation of dough in experimental baking, especially in punching and molding, is customarily regarded as one of the most difficult features to standardize. General experience has shown that there is a *personality* factor that is extremely hard to identify and measure. The work of Geddes and Goulden (1931) shows that both hand punching and hand molding contribute substantially to variability among different operators. Moen (1929) recorded similar

experience. The present report gives some consideration to these matters, as well as to the manner in which loaf characteristics are influenced by varying the punching schedule, the frequency of punches, the method of punching, and the severity of punching.

A rather extended series of experiments was also undertaken in an effort to devise a hand molding procedure that can be used by different operators with a minimum of variability. These studies were prompted by a realization of the fact that although the Type G Thomson Roll Molder serves its purpose admirably, its cost is a feature that minimizes any probability of its becoming a standard item of baking laboratory equipment.

Harrel (1926), and Herman and Hart (1927), are among those who have reported observations relating to the manner in which test loaf characteristics are affected by varying the frequency, severity, and schedule of punching the dough. Harrel's loaf volumes were the same when punching was entirely omitted as when the doughs were punched every 15 minutes, and the data of Herman and Hart confirm Harrel on this point. It will be shown, however, that the Research Fellow's recent findings in this regard were not entirely in agreement with those of Harrel and of Herman and Hart just cited.

Variations in Methods of Punching Dough

A number of variations from the specified punching procedure were studied. In each day's bake the practice was to bake half the test loaves by the *normal* specified method, and the other half by the particular variation selected. This was considered desirable in order to eliminate any variability that might be due to yeast or to unknown causes, and because, as noted in the previous report (1931, p. 287–288): "On certain days all loaf volumes tended to run higher than on other days under presumably identical conditions." These variations, however, were not ordinarily great enough to be of serious consequence; nevertheless, the precaution was taken in an effort to reduce to a minimum all variables other than the one under consideration. All doughs were machine molded unless otherwise specified.

TABLE II

NORMAL PUNCHING VERSUS NO PUNCHING

Flour number	4
Number of individuals normally treated	15
Number of individuals with no punch	15
Mean volume of normally treated loaves (cc.)	588
Mean volume of no punch loaves (cc.)	494
Standard deviation (normally treated)	10.39
Standard deviation (no punch)	7.92
Coefficient of variation (normally treated)	1.77
Coefficient of variation (no punch)	1.60

In Table II are given the results of an experiment involving 30 bakes from the same flour, 15 of which were not punched at all until molded, while the other 15 were punched normally.

Table II shows an average reduction in volume of 94 cc. caused by eliminating the first and second punch. This is a large and significant difference, and is in disagreement with the findings reported by Harrel (1926), and Herman and Hart (1927), who found that omitting the punches caused no significant difference in loaf volume. The explanation probably lies, at least partially, in differences between individual flours. Some flours are much more sensitive to variations in manipulation than others, as was repeatedly demonstrated in these studies.

TABLE III

NORMAL PUNCHING VERSUS ONE PUNCH AT 1½ HOURS

Flour number	5	6	4
Number of individuals normally punched	15	15	15
Number of individuals punched once	15	15	15
Mean volume of normally treated loaves (cc.)	588	599	618
Mean volume of abnormally treated loaves (cc.)	565	583	591
Coefficient of variation (normal series)	1.57	2.09	0.96
Coefficient of variation (one punch series)	2.75	1.70	1.14

Table III shows that when doughs were punched only once the loaf volumes ran slightly, though consistently, lower than when punched normally.

Dunlap (1926), and Herman and Hart (1927), have noted slight improvements in loaf volume by reversing the time intervals between the first and second punches. This procedure was, therefore, tried by the Research Fellow with results as recorded in Table IV.

TABLE IV

NORMAL VERSUS REVERSED PUNCHING SCHEDULE

Flour number	5	6
Number of individuals normally punched	15	15
Number of individuals with reversed punches	15	15
Mean volume of normally treated loaves (cc.)	587	582
Mean volume of reversed punch loaves (cc.)	580	592
Coefficient of variation (normally punched)	1.96	3.29
Coefficient of variation (reversed punched)	2.86	3.22

These data show no important differences between doughs punched normally and those with a *reversed* punching schedule.

A series of tests were run in which doughs were punched every 30 minutes, as compared with the *normal* specified procedure. The results of these studies are given in Table V.

TABLE V

NORMAL PUNCHING VERSUS PUNCHING EVERY 30 MINUTES

Flour number	2	5	6
Number of individuals punched normally	15	15	15
Number of individuals punched every 30 minutes	15	15	15
Mean volume of normally treated loaves (cc.)	516	595	592
Mean volume of loaves punched every 30 minutes (cc.)	526	624	612
Coefficient of variation (normally treated)	2.11	2.47	2.48
Coefficient of variation (punched every 30 minutes)	1.45	1.69	2.46

A volume differential slightly in favor of the doughs receiving an increased number of punches is shown for each of the 3 flours. The figures for variability also favor the loaves from doughs punched every 30 minutes, although the number of individuals is probably too small to justify any strong emphasis on that point.

These studies on frequency of punching show a very consistent tendency toward increasing loaf volumes with greater punching frequency, other factors being equal. The advantage of largest loaf volumes resulting from punching every 30 minutes,—if it is really an advantage,—might be regarded as of a magnitude insufficient to compensate for the extra work and inconvenience to the technician.

A study of *normal* versus *severe* punching involved experiments in which the Fellow punched half of the doughs in each series in his customary manner, but the other half of the doughs, although given the specified number of folds, were stretched further before folding and given a more vigorous treatment in all respects. The data from these studies are shown in Table VI.

TABLE VI

NORMAL VERSUS SEVERE PUNCHING

Flour number	2	4	5
Number of individuals punched normally	15	30	15
Number of individuals punched severely	15	30	15
Mean volume of normally treated loaves (cc.)	512	597	595
Mean volume of severely treated loaves (cc.)	511	597	594
Coefficient of variation (normally treated)	1.64	1.46	1.33
Coefficient of variation (severely treated)	1.76	1.59	1.97

These data show no difference between the results of *normal* and *severe* punching, when the punching was all done by one experienced operator.

A study of *punching personality* consisted of tests in which the punching was done by two different operators, but all loaves were machine molded, as usual. Operator B, although of considerable experience, was less experienced than A.

TABLE VII

DIFFERENCES IN THE PUNCHING PERSONALITIES OF TWO OPERATORS

Flour number	1	2	4	5
Number of individuals punched by operator A	30	30	30	15
Number of individuals punched by operator B	30	30	30	15
Mean volume of A's loaves (cc.)	474	515	610	608
Mean volume of B's loaves (cc.)	469	504	588	604
Coefficient of variation (A's loaves)	0.88	1.24	1.57	2.06
Coefficient of variation (B's loaves)	1.48	1.88	2.93	2.16

The data in Table VII show slightly, though consistently, larger average loaf volumes for the doughs punched by operator A than for those punched by operator B. Furthermore, operator A showed decidedly less variability than operator B. A brief series of experiments was also made for the purpose of determining whether or not increasing the frequency of punches would tend towards a reduction of variability between operators A and B. In this study each operator punched the doughs every 30 minutes, with the results as shown in Table VIII.

TABLE VIII

EFFECT OF INCREASED FREQUENCY OF PUNCHING ON VARIABILITY BETWEEN OPERATORS

Flour number	5	6
Number of individuals punched by operator A	15	15
Number of individuals punched by operator B	15	15
Mean volume of A's loaves (cc.)	583	600
Mean volume of B's loaves (cc.)	581	609
Coefficient of variation (operator A)	2.19	1.93
Coefficient of variation (operator B)	2.51	2.30

Here is shown close agreement between the results of A and B when the frequency of punching is increased. However, much further study on this point would be necessary in order to definitely determine whether the advantages of more frequent punching are sufficient to justify the extra work involved.

The possibility of substituting machine punching for the usual hand manipulation has frequently been discussed among laboratory baking technologists, and doubtless there are operators who habitually use some sort of mechanical device for punching doughs. On the other hand there are many who prefer hand manipulation for the obvious reason that it offers a better basis for acquiring information regarding the physical properties of the dough.

Experiments were undertaken for the purpose of comparing the prescribed hand punching procedure with punching by merely running the dough through the Thomson Roll Molder at the time intervals specified for the first and second punch. The results of this study are shown in Table IX.

TABLE IX

USE OF MECHANICAL MOLDER FOR PUNCHING DOUGHS

Flour number	2	3	5
Number of individuals hand punched	15	15	15
Number of individuals machine punched	15	15	15
Mean loaf volume of hand punched loaves (cc.)	512	617	599
Mean loaf volume of machine punched loaves (cc.)	530	600	605
Coefficient of variation (hand punched)	1.55	1.62	1.89
Coefficient of variation (machine punched)	1.86	1.59	2.00

These data show no reason why the Thomson Roll Molder should not serve to advantage for punching as well as for molding doughs. As previously suggested, however, the cost of the machine and the fact that some individuals prefer to feel the dough while working it by hand are important factors. It is to be noted that the machine punching gave a slightly increased loaf volume with flour No. 2, but affected a loaf volume reduction of equal magnitude with flour No. 3.

Molding Studies

The writers believe the performance of the Thomson Model G Roll Molder to be highly satisfactory and efficient for the type of work here under consideration. It is safe to say that its general adoption and use, both for molding and punching doughs would greatly reduce variability among different technicians. Nevertheless, it would be advantageous if some simpler and less costly device or method could be devised that would accomplish essentially the same purpose. This should be within the range of possibility, and with this end in view there have been undertaken some more or less detailed studies of certain factors involved in hand molding, and in the use of relatively simple mechanical devices that can be used in conjunction with hand manipulation.

Since hand molding is perhaps the general practice in the laboratories of the majority of cereal technologists at the present time, one of the first steps in this phase of the work was an inquiry into the general nature of the effects produced by certain variations and modifications in the hand-molding technique of an experienced operator. The following experiments were undertaken:

EXPERIMENT 1. The standard method versus molding by means of a series of folds on a glass surface with no dusting flour (the latter procedure was the one to which the Baking Fellow was most accustomed). The results obtained in this experiment suggest that an experienced operator is likely to get the same results by one type of hand molding as by another.

Flour number	5	6
Number of individuals molded by each method	15	15
Mean volume by standard method (dough rolled up) (cc.)	560	597
Mean volume by folding procedure (cc.)	559	601
Coefficient of variation (standard procedure)	3.34	2.06
Coefficient of variation (folding procedure)	3.38	1.69

EXPERIMENT 2. Molding on a glass surface without dusting flour versus molding on a wooden surface with dusting flour. Molding was accomplished by a series of folds.

Flour number	5
Number of individuals	15
Mean loaf volume without dusting flour (cc.)	579
Mean loaf volume with dusting flour (cc.)	566
Coefficient of variation (without dusting flour)	3.74
Coefficient of variation (with dusting flour)	3.19

EXPERIMENT 3. The conditions were the same as Experiment 2, but the standard hand procedure (rolling up of the dough) was used.

Flour number	6
Number of individuals used for each series	15
Mean loaf volume without dusting flour (cc.)	575
Mean loaf volume with dusting flour (cc.)	559
Coefficient of variation (without dusting flour)	3.95
Coefficient of variation (with dusting flour)	3.33

The results obtained in Experiments 2 and 3 indicate a slight reduction of loaf volume when dusting flour is used in hand molding procedure.

EXPERIMENT 4. Molding by the standard procedure on a glass slab versus molding on large piece of cotton belting as suggested by G. Moen.

Flour number	2
Number of individuals for each series	15
Mean loaf volume when molded on glass (cc.)	510
Mean loaf volume when molded on belting (cc.)	500
Coefficient of variation (molded on glass)	3.13
Coefficient of variation (molded on belting)	2.91

There were no very significant differences, either in volume or in variability by the two methods. Molding on belting appears less likely to chill doughs in cold weather.

EXPERIMENT 5. Flour No. 6. The effect of hand molding on plain cotton belting as compared with belting which previously had grease and flour rubbed into it was studied.

The grease treatment of the belt reduced the average loaf volume of flour No. 6 from 549 to 522 cc.

Experiments 1 to 5, while neither conclusive nor exhaustive, suggest nevertheless that the present standard hand molding method is perhaps as suitable as any procedure that is exclusively a hand

method. A piece of heavy cotton or canvas belting constitutes a very satisfactory molding surface.

The next phase of the molding experiments involved attempts to improve upon the hand manipulation, in the interests of uniformity among different operators. This portion of the work necessitated the cooperative efforts of several technicians, and was, therefore, participated in by 3 collaborators designated as A,[4] B,[5] and C.[6] All three were experienced in the actual technique of laboratory baking by the A. A. C. C. method; operator A being the most experienced, B next, and C the least.

The flour used for the greater part of the preliminary collaborative molding studies was flour No. 6, a flour of exceptionally high protein content and having a potentiality for producing loaves of large volume. In the first series of these studies all doughs were mixed and punched by operator A. Each of the 3 operators molded 10 out of the total of 30 loaves baked per day.

Among the factors that were studied with reference to their influence upon bread characteristics, in addition to their effects on variability among the 3 operators, were the following: Flattening the dough and rolling it up according to the prescribed method versus molding by a series of folds; severe versus light treatment in pounding out the gas; loose versus tight rolling up of the dough; nature of the surface upon which dough was molded; the use of a rolling pin and *wooden guides* or *tracks* with several modifications; different punching procedures in relation to molding by different operators; the use of sheeting rolls only, for flattening out the dough, with subsequent rolling up by hand; and, the influence of different types of molding on the characteristic symptoms produced by potassium bromate.

EXPERIMENT 6. Standard molding method. Flour No. 6. Molding by 3 operators; all other operations done by A.

Operator	A	B	C
Number of individual loaves	10	10	10
Average loaf volume (cc.)	609	557	549
Coefficient of variation	3.51	4.28	2.01

The crumb properties in the loaves baked by operator A showed the most symptoms of age. A's loaves also were of much greater volume than those of operators B or C.

EXPERIMENT 7. This experiment was carried out the same as was Experiment 1 with the exception that the doughs were pounded out more severely and rolled up more tightly.

[4] A—P. P. Merritt.
[5] B—R. M. Sandstedt.
[6] C—M. J. Blish.

Operator	A	B	C
Number of individuals	10	10	10
Average loaf volume (cc.)	554	525	510
Coefficient of variation	3.94	3.23	1.68

The loaf volumes in all cases were lower, but of the same order as in Experiment 6. Operator C produced loaves of the lowest volume, but he had, on the other hand, the lowest coefficient of variation. This was likewise true in Experiment 6.

EXPERIMENT 8. The conditions were the same as in Experiments 6 and 7, but the molding was done by series of *folding operations* instead of by the standard method. A wooden molding board and dusting flour were used.

Operator	A	B	C
Number of individuals	10	10	10
Mean loaf volume (cc.)	608	563	539
Coefficient of variation	2.09	2.84	3.13

The differences in molding personality are similar to those experienced in Experiments 6 and 7.

EXPERIMENT 9. The conditions were the same as in Experiment 8, but the molding was done on a large piece of cotton belting instead of on wood.

Operator	A	B	C
Number of individuals	10	10	10
Mean loaf volume (cc.)	589	556	556
Coefficient of variation	3.31	3.41	4.05

There was a tendency for slightly more concordant results between operators. Cotton belting, as suggested and used by G. Moen, makes a satisfactory molding surface. The doughs do not stick, in fact show a tendency to contract rather than to stay flattened out during molding. Operator A still produced loaves of the greatest volume.

EXPERIMENT 10. The gas was knocked out of the doughs by passing through the sheeting rolls, only, of the Thomson Roll Molder. The strip of dough was then rolled up *lightly* by hand, using a belting cloth molding surface.

Operator	A	B	C
Number of individuals	10	10	10
Mean loaf volume (cc.)	591	584	577
Coefficient of variation	2.22	2.78	2.46

There was a decided improvement in uniformity between the different operators. Operator A's loaf volumes were now only slightly larger than B's and C's. The indications are that molding variations among operators may be due more to personality in pounding out gas rather than to the manner of rolling up the sheet of dough. This suggested the use of a rolling-pin and two wooden tracks as a simple means whereby different operators might flatten out dough to uniform thickness, with uniform pressure and uniform gas removal.

Some experiments were, therefore, undertaken in which the ball of dough was removed from the bowl at molding time and placed, with wet side down, between two parallel wooden strips about 8 inches apart. A rolling pin was then placed on the top center of the dough mass, pressed down until it touched both tracks and given one roll each way from the center. The two opposite sides of the flat circular sheet of dough were then overlapped as in photograph 2, Cereal Chemistry, Volume V, page 160, and the dough again placed between the wooden strips, (parallel with them) and rolled out flat as before. The strip of dough was then coiled or rolled up by hand. With some flours a light application of dusting flour to the rolling pin was found necessary.

EXPERIMENT 11. The dough was flattened out with a rolling pin between wooden strips $\frac{3}{16}$ inch thick, as described in the preceding paragraph, and rolled up loosely, without pressure or stretching.

Operator	A	B	C
Number of individuals	10	10	10
Mean loaf volume (cc.)	573	571	556
Coefficient of variation	2.65	4.98	2.74

The doughs tore badly. Apparently the $\frac{3}{16}$ inch thickness of the wooden tracks was not deep enough. However, a decrease was noted in loaf volume variability among operators.

EXPERIMENT 12. The conditions were the same as in Experiment 11, but the thickness of the wooden tracks was increased to $\frac{5}{16}$ inch, and the doughs were rolled up tightly.

Operator	A	B	C
Number of individuals	20	20	20
Mean loaf volume (cc.)	600	591	587
Coefficient of variation	2.08	1.76	1.89

There was no tearing of the dough. Loaf volumes were larger than in Experiment 12. There was uniformity among the 3 operators

with regard to loaf volume and crumb structure. Grain and cell structure were more open than was the case when either the standard procedure or machine molding was tried.

EXPERIMENT 13. The conditions were similar to those in Experiments 11 and 12 with the exception that the first rolling operation was with tracks of $\frac{3}{16}$ inch thickness, and the second operation with tracks $\frac{5}{16}$ inch deep.

Operator	A	B	C
Number of individuals	10	6	10
Mean loaf volume (cc.)	592	602	595
Coefficient of variation	2.67	3.44	4.33

This procedure offered no advantages over that described in Experiment 12. Individual variability was high.

EXPERIMENT 14. The conditions were the same as described in Experiment 12. Flour No. 2, however, was used in place of flour No. 6.

Operator	A	B	C
Number of individuals	10	10	10
Mean loaf volume (cc.)	513	516	517
Coefficient of variation	2.01	2.57	1.83

Uniformity among operators was good. Individual variability was satisfactory.

EXPERIMENT 15. The conditions were the same as in Experiment 14, but using flour No. 5.

Operator	A	B	C
Number of individuals	10	10	10
Mean loaf volume (cc.)	569	582	591
Coefficient of variation	2.49	2.03	2.36

It is apparent that *personality* in hand molding can be greatly reduced by the rolling pin procedure. Here, as in Experiment 14, the loaf volume results obtained by operator A fell below those obtained by operators B and C.

EXPERIMENT 16. The conditions were the same as in Experiments 12, 14, and 15. However, the final rolling-up by hand was done less tightly. Effort was made to roll-up the dough firmly, but neither too tight nor too loose. Flours No. 2, 5 and 6 were used.

Operator	A	B	C
Results with flour No. 2			
Number of individuals	10	10	10
Mean loaf volume (cc.)	535	530	516
Coefficient of variation	1.86	2.28	2.31
Results with flour No. 5			
Number of individuals	10	10	10
Mean loaf volume (cc.)	607	604	598
Coefficient of variation	1.19	0.96	1.25
Results with flour No. 6			
Number of individuals	10	10	10
Mean loaf volume (cc.)	613	601	599
Coefficient of variation	1.53	1.22	2.40

A slightly increased loaf volume was obtained as compared with the dough rolled up tightly.

There can be no doubt that the rolling-pin method as described for Experiment 16 very effectively reduced variability in hand molding among different operators where all other factors were equal. The procedure is convenient and easy to follow. Comparatively speaking, loaves of good volume are obtained. Loaves molded by this procedure usually give a more open grain and cell structure than loaves molded either by the standard hand method or by the Thomson Roll Molder. Variations in crumb structure among different flours apparently do not register as sharply by this method as by the standard method. Whether or not this constitutes a valid basis for serious objection is a controversial matter.

Collaborative Punching Studies Combined with the Rolling-Pin Method of Molding

Since the above described rolling-pin method as applied to hand molding was found to be very effective in reducing molding variability among individual operators, it was deemed advisable to extend the collaborative studies in an effort to answer the following question: Will this rolling-pin molding procedure operate toward a reduction of variability caused by the personal factor in *punching* doughs? Accordingly, a series of experiments were initiated in which each individual operator not only molded his doughs by the rolling-pin method, but punched them as well.

EXPERIMENT 17. The doughs were molded as in Experiment 16, but were punched by each operator according to the standard procedure.

Operator	A	B	C
Results with flour No. 2			
Number of individuals	35	35	20
Mean loaf volume (cc.)	529	533	521
Coefficient of variation	1.54	2.09	1.81
Results with flour No. 5			
Number of individuals	40	40	40
Mean loaf volume (cc.)	589	578	574
Coefficient of variation	1.69	1.83	2.11
Results with flour No. 6			
Number of individuals	20	20	20
Mean loaf volume (cc.)	627	619	613
Coefficient of variation	2.77	1.91	1.98

The widest spread in average loaf volume among the three operators was 15 cc. (flour No. 5, operators A and C). The tendency for the most experienced operator to have the largest loaf volume still persists, but the differential is no longer as serious as was the case in Experiment 6. The 3 operators checked closely in both external and internal loaf characteristics. The values for coefficient of variation indicate a reasonably good concordance among the results of each individual operator.

In addition to the studies included in Experiment 17, a number of collaborative tests were made involving a variety of modifications in punching technique. These variations included: (1) Knocking down the dough without removing from the bowl; (2) punching with the rolling pin; (3) reducing the number and severity of folds; (4) increasing the number and severity of folds; (5) changes in punching schedule; (6) changes in punching frequency; and, (7) punching by running through the mechanical molder.

Most of these modifications gave results that were inferior to those shown in the results given for Experiment 17, and since a presentation of the data would needlessly take up space without serving any useful purpose it is omitted. The one interesting exception is shown in Experiment 18.

EXPERIMENT 18. The doughs were punched every 30 minutes, each punch consisting of 5 folds. Flour No. 6 was used and rolling-pin molding carried on as described for Experiment 17.

Operator	A	B	C
Number of individuals	10	10	10
Mean loaf volume (cc.)	677	661	672
Coefficient of variation	1.82	1.50	3.71

Loaf volumes were much larger than usual. The agreement among operators was excellent. The results substantiate and supplement the data shown in Tables V and VIII.

The experimental results presented in Experiment 17 seemed promising enough to warrant collaborative trials with a variety of baker's flours. The results are given as Experiment 19.

EXPERIMENT 19. Twelve baker's flours used. They were baked in duplicate by each of the 3 operators. Each operator punched his own doughs by the standard procedure and molded them by the rolling-pin method as used in Experiment 17. The results follow:

Flour number	Loaf volume obtained by		
	Operator A	Operator B	Operator C
	cc.	cc.	cc.
10	572	566	562
8	440	461	455
15	482	496	501
16	467	467	465
17	488	496	491
18	524	518	516
37	483	488	459
12	461	476	492
13	523	524	515
9	559	556	574
14	526	517	517
7	616	611	567
Average	512	515	510

With all of the 12 flours excellent agreement with regard to loaf volume was obtained by the 3 operators with the exception of operator C's low volume on flour No. 7. Other loaf characteristics checked well.

Comments and Conclusions on Rolling-Pin Molding Method

During the course of the experiments on rolling-pin molding, numerous minor modifications of the procedure were considered. Most of these dealt with variations in methods of sealing the ends and seam of the molded dough, and with certain minor variations in methods of panning the dough. These were found to be for the most part of no serious importance.

The details of the procedure as finally adopted, and as used in Experiments 16, 17, and 18, are as follows:

Two narrow strips of wood $\frac{3}{8}$ inch thick are placed upon the molding surface about 8 inches apart, parallel, and pointing toward the operator. The ball dough is removed from the fermentation bowl without any "knocking down," and placed between the wooden strips, wet side down. The rolling-pin, pressed down on the

top center of the dough until contact is made with both of the wooden strips, is given one roll each way from the center, rolling the dough into a flat circular sheet. The dough is turned over, and the two opposite sides are overlapped in such a manner as to form a roll of dough as in photograph 2, Cereal Chemistry, Volume V, page 160, with one end of the roll slightly thicker than the other. This is again placed between the wooden strips, parallel with them with the seam down, and rolled out into a flat strip of dough which is slightly narrower at the end fartherest from the operator. The dough is once more turned over and brought to a *relaxed* or unstretched condition. It is then rolled up, starting from the narrower end, and rolling toward the operator, with no attempt either to stretch the dough or to use the thumbs for "packing in" the dough during the rolling process. The roll of dough is lightly sealed *on the side only*, with the thumb and fingers, and placed in the pan with the seam down. The ends of the roll are then sealed by pressing down with the fingers at each end of the pan.

Based upon the data presented in Experiments 16, 17, and 19, as well as upon various observations made during this phase of the work, the following tentative conclusions are in order:

1. The rolling-pin method of molding tends toward far greater concordance among the results of different technicians than any other method in which both punching and molding are done chiefly by hand and without recourse to specialized mechanical contrivances.

2. The method gives loaves having, as a rule, a more open and spherical crumb structure than loaves molded either by the standard method or by the Thomson Roll Molder.

3. The method tends to minimize variations in crumb structure among different flours,—variations that are slightly more prominent in other more commonly used methods of molding. Many will consider this a disadvantage.

4. The method is simple, convenient, and easy to operate, and requires a minimum of skill and experience.

That the uniformity of results among operators A, B, and C, when using the prescribed rolling-pin molding procedure, is no guarantee that the method would effect a similar concordance among workers in *different* laboratories, is duly recognized. Operators A, B, and C, working together, had an opportunity to standardize their manipulation to a far greater degree than would be true of workers in different laboratories; nevertheless, the method shows encouraging possibilities. In the interests of standardization, and until some generally acceptable mechanical process is devised and approved, it may be worthy of collaborative study involving different laboratories.

The experiments on increasing the frequency of punching, recorded in Tables V and VIII, and in Experiment 18, indicate that this subject too is perhaps worthy of further study in spite of the fact that more frequent punching involves a little extra manipulation.

Use of The " S-Rolls " in Punching and Molding

The substitution of mechanical devices for hand manipulation in punching and molding has frequently occurred to cereal technologists, and there is no doubt that this would be a rational step in the interests of standardization. Thus far, however, no generally acceptable mechanical contrivance has appeared. The possibilities of the Thomson Roll Molder have been indicated in Table IX, but the size and cost of this piece of equipment, especially the cost, serve as a serious stumbling block. However, the possibility of using sheeting rolls, alone, is indicated by Experiment 10.

A set of sheeting rolls, operated by hand, is neither complicated nor an excessively expensive item of equipment. Accordingly, a set of these rolls was devised and built in the shop of a local mechanic. The design and construction of these rolls, which are herein designated as the "S-rolls," are clearly shown in Figures 1 and 2. The roll dimensions, considering only the working surfaces, are $2\frac{1}{2}$ inches in width by $3\frac{1}{2}$ inches in diameter. The flanges on rear roll are $\frac{5}{16}$ inch wide and $\frac{7}{16}$ inch deep. There is a device for adjusting the distance between the rolls within a rather narrow range.

After preliminary trials and experiments with the S-rolls, the following procedure was established and used throughout this phase of the work:

For the first punch the dough is removed from the bowl with a flexible spatula, with no attempt to knock out any gas during the operation. The wet side of the dough is lightly sealed over by drawing the dry edges together with thumb and fingers. The dough is introduced into the rolls with the left hand while revolving them as rapidly as possible with the right hand. The left hand is also used for catching the strip of dough as it emerges from the under side of the rolls. It is desirable to catch the strip of dough before it crumples up, and the ability to do this is easily acquired with a little practice. The rolls should be turning as rapidly as conveniently possible before the dough is introduced, in order to minimize chances of sticking to the rolls. For normal doughs very little trouble with sticking has been encountered, when handled in the prescribed manner. For abnormally sticky doughs, it is advisable to rub the rolls with a very lightly greased cloth before introducing the dough. A very light greasing of this character has been found to have no observable effect upon loaf properties. At the first punch the strip of dough, after going through the S-rolls, is lightly rolled up and returned to the pan without any special sealing or rounding up into a spherical shape. The second punch is merely a duplication of the first. The molding procedure is a duplication of the punching operation, excepting that the roll of dough is sealed on the side, only, and placed in the pan with the seam down. The ends are then sealed by pressing down at each end of the pan with the fingers. As a matter of fact the *sealing* of the dough, either on the side or on the ends, or both, has not been found to be a critical factor either in molding with the rolling pin or with the S-rolls.

Having established the procedure just described, some experiments were conducted for the purpose of deciding upon the optimum adjustment of the space between the two rolls. As a result of these experiments, $\frac{1}{4}$ inch spacing was adopted.

Typical effects of varying roll settings are shown in the following study.

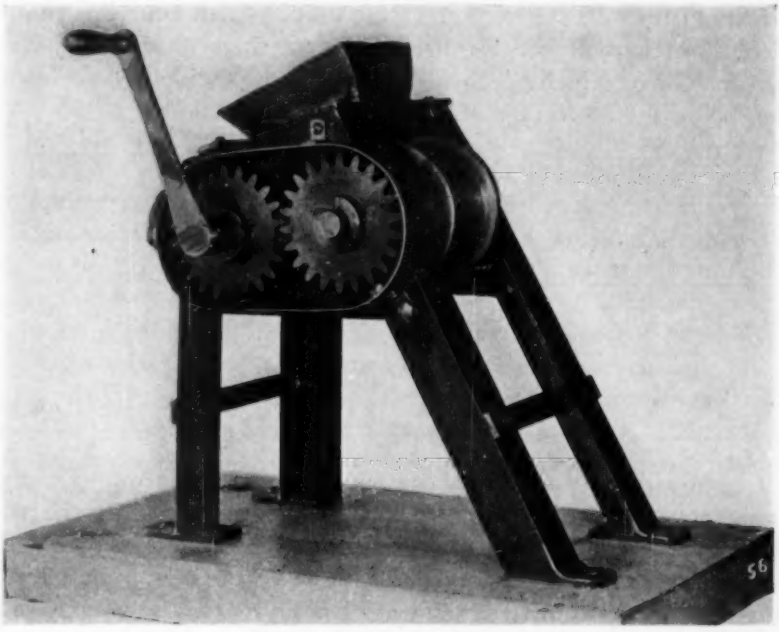

Fig. 1. Horizontal view of S-roll.

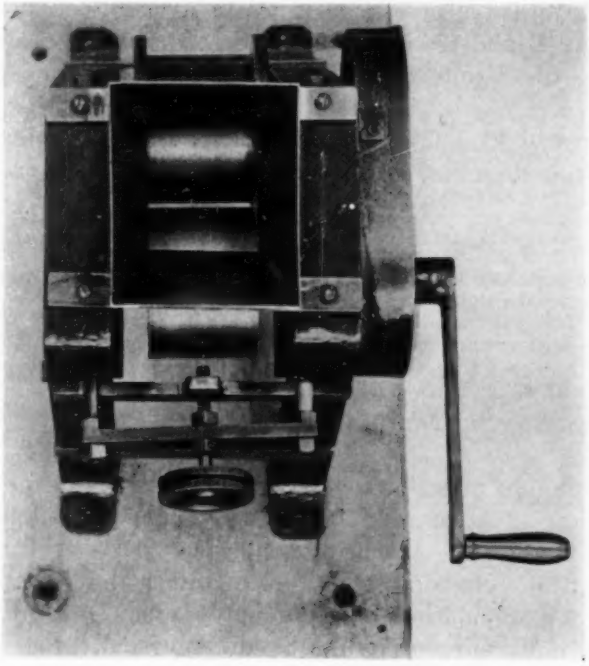

Fig. 2. Vertical view of S-roll.

EXPERIMENT 20. Five flours were used. Each flour was run at 3 different settings of the S-rolls. These settings provided distances between rolls of $\frac{1}{4}$ inch, $\frac{5}{16}$ inch and $\frac{3}{8}$ inch, respectively. Each value given below is the average of duplicate determinations.

Flour number	Loaf volume		
	Roll setting		
	$\frac{1}{4}$ inch	$\frac{5}{16}$ inch	$\frac{3}{8}$ inch
	cc.	cc.	cc.
5	573	555	552
7	635	607	577
9	540	530	535
17	512	497	490
8	507	477	482

Crumb structure was fairly compact and very uniform. There was a tendency for a slightly more open cell structure with the wider settings. With the $\frac{1}{4}$ inch setting, there was also a tendency for larger loaf volumes although differential volumes were less pronounced in some flours than in others. Thus, for flour No. 7, the volume differential between the $\frac{1}{4}$ inch and the $\frac{3}{8}$ inch setting was 58 cc., while with flour No. 9, there was no significant volume differential whatever.

The next order of investigation was a series of collaborative tests by operators B and C [7] involving replicated test bakes on the same flour, using the S-rolls in accordance with the previously described procedure.

EXPERIMENT 21. Each operator baked a series of loaves on the same day from flour No. 5, using the S-rolls for punching and molding.

Operator	B	C
Number of individuals	14	14
Mean loaf volume (cc.)	573	569
Coefficient of variation	1.57	1.29

There was satisfactory agreement between operators. Individual variation was low. Uniform porosity of crumb was a very prominent feature.

The issue now arises as to which of the 4 types of molding under consideration is best adapted to the requirements of a standard experimental baking test. Manifestly, no final decision can be made without further collaborative studies involving different laboratories. Mechanical manipulation unquestionably offers the greatest possibilities for uniformity of results by different individual technicians.

[7] These tests were made after the resignation of operator A, P. P. Merritt.

Considering the matter from all angles, the writers, at present, give preference to the "S-rolls" type of manipulation. The use of the S-rolls appears to greatly minimize the personality factor, while at the same time it does not eliminate opportunities to get the *feel* of the dough. The cut sections of loaves molded by the S-rolls almost invariably show greater uniformity of porosity, grain, and cell structure than loaves molded by any of the other procedures.

What the comparative loaf volumes and external loaf characteristics are as influenced by the various types of molding procedure, and in what manner the various types of molding affect the characteristic symptoms produced by oxidizing agents, are some of the items which have been considered in experiments hereinafter recorded. Some comparative external and internal characteristics produced by the 4 types of molding are shown in Figures 3 and 4.

Table X records loaf volume measurements resulting from applying all 4 molding procedures, respectively, to each of 8 different flours.

TABLE X

COMPARATIVE LOAF VOLUMES BY DIFFERENT MOLDING METHODS

Flour number	Standard method	Thomson roll molder	Rolling-pin method	S-rolls method
	cc.	cc.	cc.	cc.
6	545	580	560	570
2	530	535	500	532
5	545	585	555	560
9	540	547	555	527
7	595	635	593	652
10	595	580	551	527
17	505	530	475	495
8	475	467	460	522

The data in Table X show that, in general, the loaf volumes resulting from the different flours vary in essentially the same order regardless of the molding procedure. Certain inconsistencies, however, are noticeable. Thus, flour No. 8 gives a much larger loaf volume by the S-rolls method than by the Thomson Roll Molder. Quite the reverse is true with flour No. 10. Figure 5 shows graphically a series of comparisons made between results obtained from 15 miscellaneous baker's flours by the S-rolls method and by the Thomson Roll Molder. Each point along the horizontal axis denotes an individual flour sample. It is apparent that both graphs in Figure 5 show the same general conformation, which means that the two methods, when applied to miscellaneous flour samples, will show volume differentials of the same general order of magnitude.

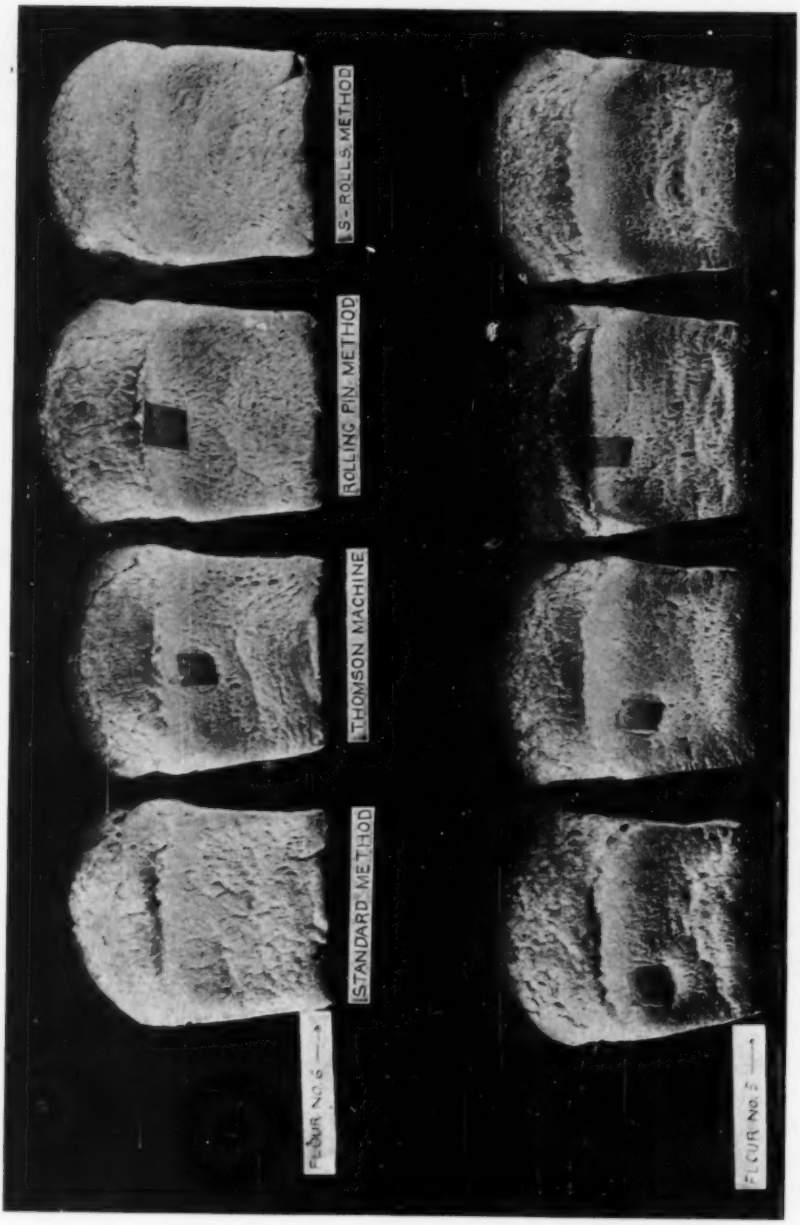

Fig. 3. External appearances of loaves molded by four types of loaf molding devices.

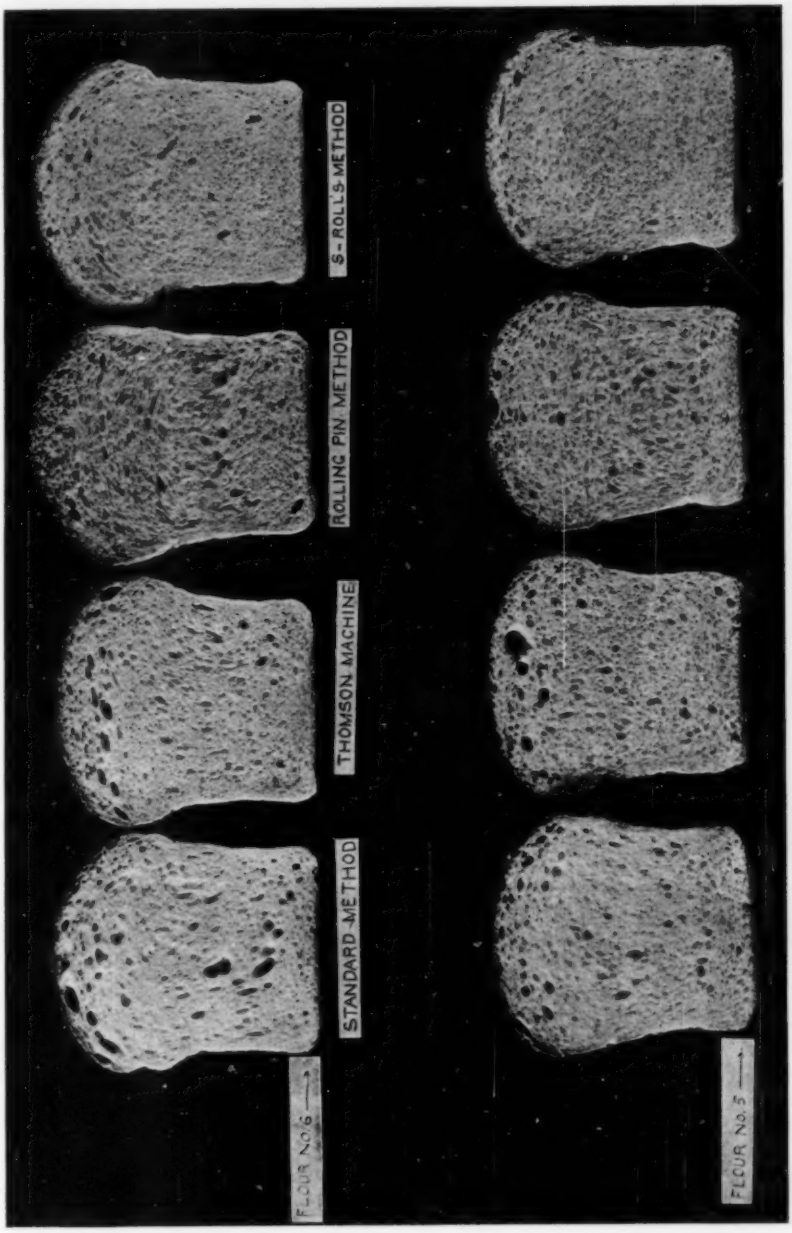

Fig. 4. Internal appearances of loaves molded by four types of loaf molding devices.

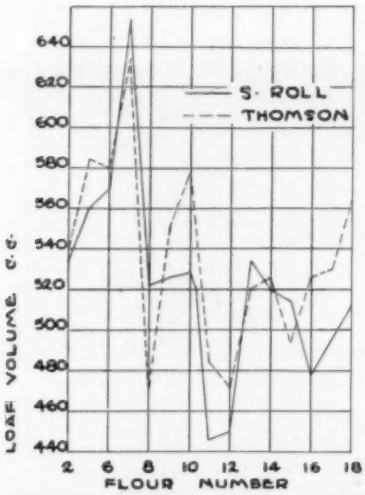

Fig. 5. Comparison of loaf volumes obtained from the baking of 15 baker's flours using the Thomson Roll Molder and the S-roll.

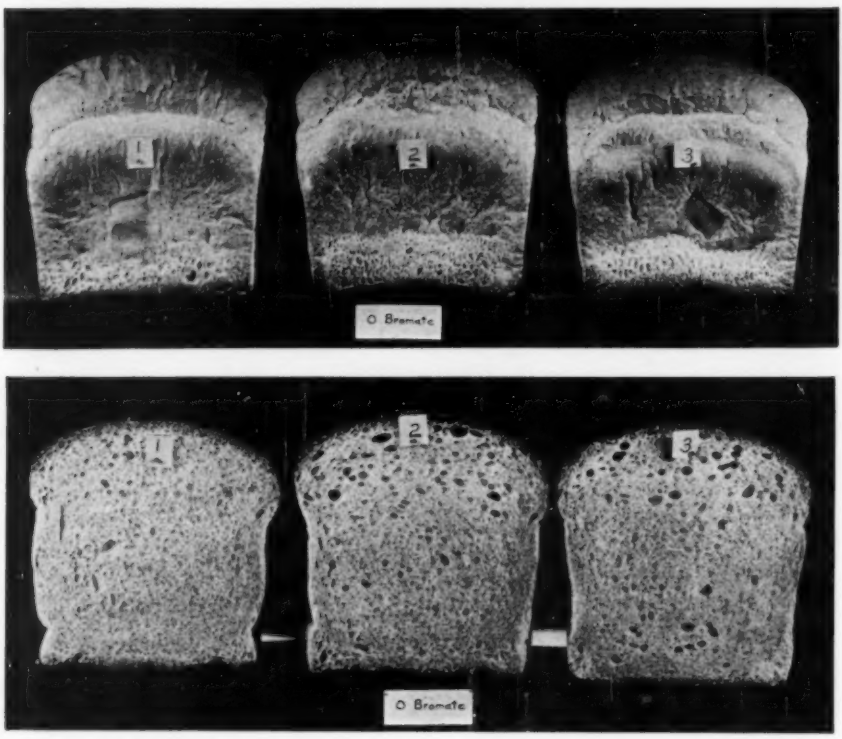

Fig. 6. Effect of molding procedure on bromate differential test—no bromate used.

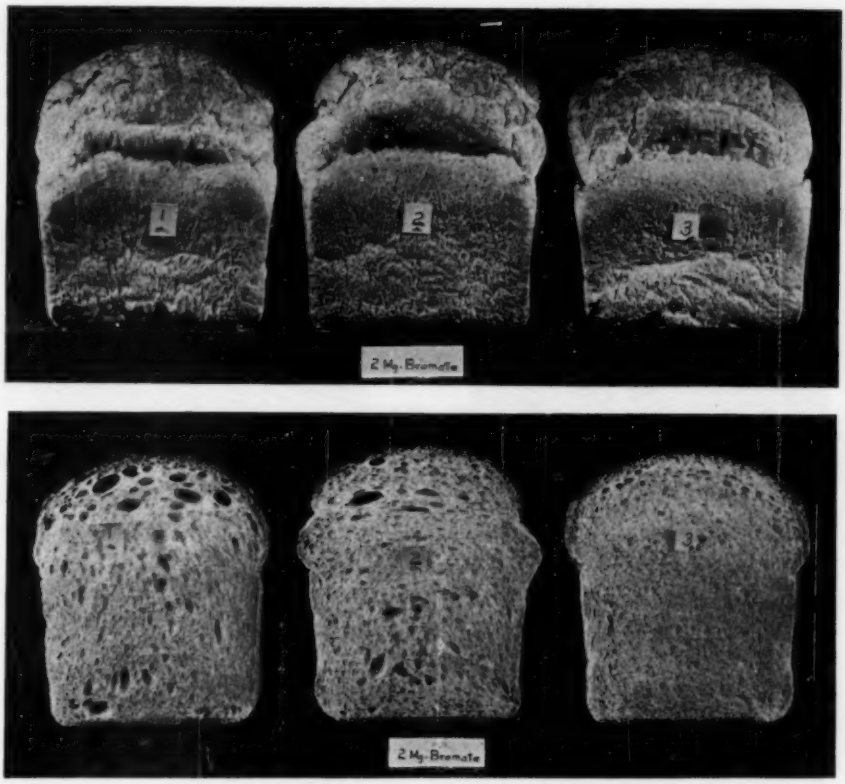

Fig. 7. Effect of molding procedure on bromate differential test—2 mgs. bromate used.

Table XI shows the comparative effects of 3 methods of molding upon the manner in which a flour may be expected to respond to the use of certain oxidizing agents. For this experiment a freshly milled unbleached clear was used, and a bromate differential series was baked using each of 3 molding procedures.

TABLE XI

EFFECT OF MOLDING PROCEDURE ON BROMATE DIFFERENTIAL TEST

Molding method	Milligrams of $KBrO_3$				
	0	1	2	3	4
	Loaf volume				
	cc.	cc.	cc.	cc.	cc.
Standard method	532	558	592	565	475
Rolling-pin method	505	575	615	612	550
S-rolls method	473	560	615	613	597

Table XI suggests that the method of molding is a very important factor in the estimation of a flour's bromate tolerance. All showed

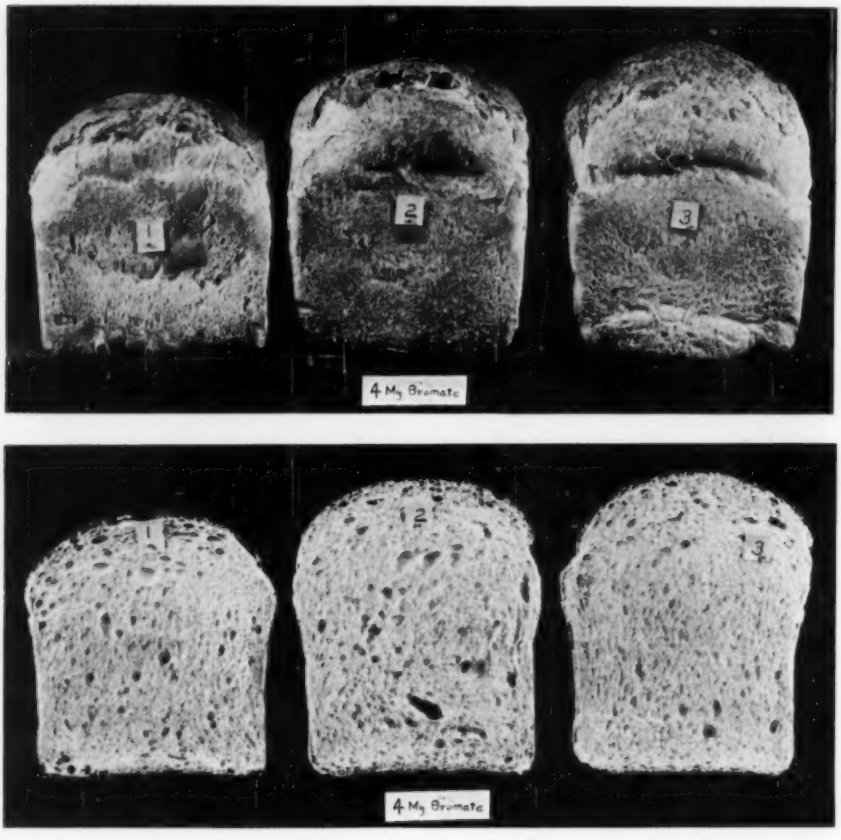

Fig. 8. Effect of molding procedure on bromate differential test—4 mgs. bromate used.

nearly the same volume stimulation with 1 and 2 mg., respectively, of $KBrO_3$, but with larger quantities the hand molded loaves *broke down* to a far greater extent under comparable conditions than did the S-rolls molded loaves. The external and internal characteristics of this series are shown photographically in Figures 6, 7, and 8. In these photographs, loaves numbered 1 were hand molded by the standard method; numbers 2, by the rolling-pin method; and numbers 3, by the S-rolls. Here again are seen the typical differences in crumb characteristics, as produced by the different molding procedures.

Absorption Studies

Harrel (1926), and Herman and Hart (1927), as well as many others, have reported that absorption is a factor of importance in its effect upon loaf properties. Merritt (1931) cited a brief experiment dealing with this matter, and since then a more thorough study of the

question has been made involving several flours. These studies have covered an absorption range of from 54% to 74%, and they have also taken account of the effect of varying the absorption upon tolerance to different degrees of mechanical mixing. The data resulting from these experiments on 4 flours are set forth graphically in Figures 9 to 12, inclusive. Each of the 4 flours showed a pronounced tendency toward maximum loaf volume at approximately 66% absorption. This is in close agreement with the results obtained by Harrel (1926). Response to prolonged mixing does not appear to have been seriously dependent upon the absorption. Three of the 4 flours showed a positive volume response to extra mixing, but the general relation between absorption and loaf volume remained practically the same regardless of the mixing time employed.

Practically all flours used in the Research Fellow's experiments have worked best in dough at higher absorptions than that prescribed in the present specifications for the basic procedure (58% on 15% moisture basis). For most flours, that have been handled in this work, an average absorption of approximately 61% on a 15% moisture basis would seem more appropriate. This may be partially due to machine mixing.

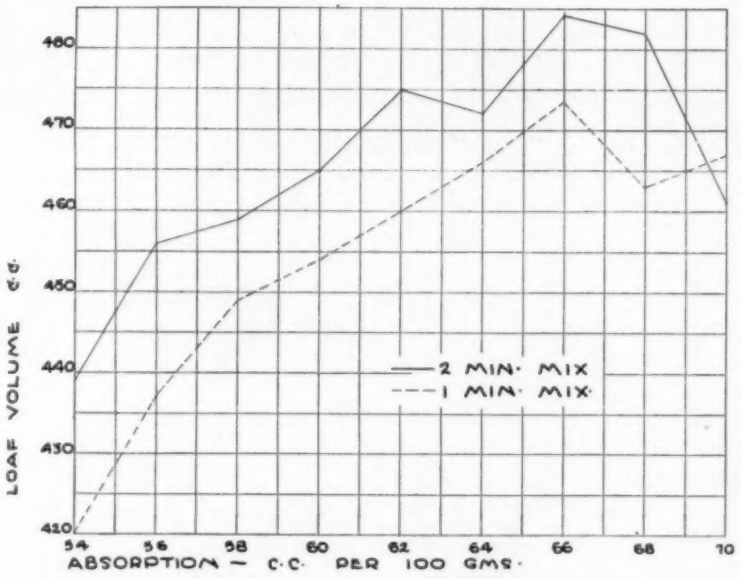

Fig. 9. Relation of absorption to loaf volume with flour No. 1.

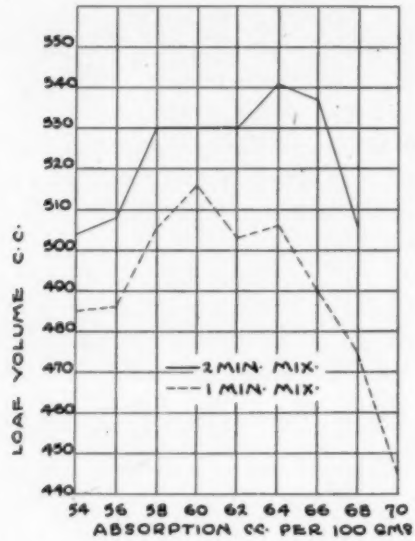

Fig. 10. Relation of absorption to loaf volume with flour No. 2.

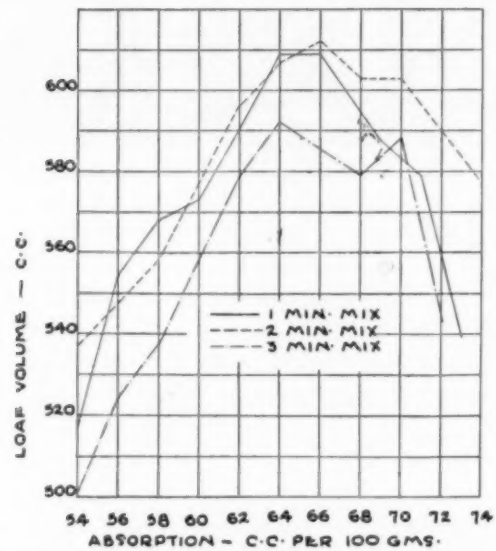

Fig. 11. Relation of absorption to loaf volume with flour No. 5.

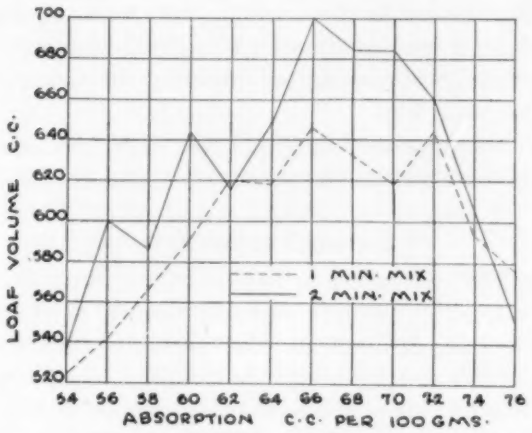

Fig. 12. Relation of absorption to loaf volume with flour No. 6.

Fermentation Bowl Studies

Among the numerous factors studied by Herman and Hart (1927) was the shape of the fermentation bowl. In their experience it made very little difference whether the dough was fermented in low or in tall formed bowls, with the exception of a slight difference in crumb color in the loaves.

In this work a similar study was made using 3 different flours, and comparing the regulation specified oatmeal bowls with tall formed, liter beakers. The results of this study are shown in Table XII.

TABLE XII

Effect of Shape of Fermentation Bowl on Baking Results

Flour number	2	5	6
Number of individuals in each series	15	15	15
Mean loaf volume using regulation bowls (cc.)	506	568	587
Mean loaf volume using tall beakers (cc.)	484	551	549
Coefficient of variation (regulation bowls)	1.56	1.70	3.13
Coefficient of variation (tall beakers)	1.74	1.89	3.19

This table shows that the loaf volume values were in all 3 cases significantly in favor of the prescribed low form bowls. There were no appreciable differences in the internal characteristics that could be attributed to differences in shape of fermentation bowl.

Covered Fermentation Bowls. Thirty tests with flours No. 5 and 6, respectively, were made, in which the humidity in the fermentation cabinet was lowered to that of the room, and the doughs were fermented in the same tall form beakers as in the experiments recorded in Table XII. The beakers were covered with watch glasses. The average loaf volume for flour No. 5 was 544 cc., as compared with

549 cc. when fermented in the same beakers without covers. These differentials, though very small, are quite possibly due to the fact that the doughs crusted slightly during proofing on account of the low humidity in the proof box.

All things considered, there appears to be no justification for changing the present specifications regarding the type of fermentation bowls.

Baking Pan Studies

With regard to baking pans, the following items have long been recognized as having distinct and significant influence on bread properties: Size, shape, material, and greasing versus non-greasing. The selection of the particular pan best suited to all requirements of the standard baking test is not as simple a matter as might at first be supposed.

A number of workers, including Harrel (1926), Herman and Hart (1927), Lewis and Whitcomb (1928), and Whiting (1929), have reported upon various aspects of the baking pan situation. The studies of these and of other investigators indicate that low sided pans give larger loaf volumes, comparatively, than high sided pans; that greasing pans reduces loaf volume; and that the shape of the pans has considerable bearing upon loaf characteristics.

There is no doubt of the desirability of reaching a general agreement upon some standard baking pan. In addition to giving dimensions, present specifications call for 4XXXX spotless metal—25 gauge, 0.55 mm. thick, which requires no greasing. The selection of this material is based, no doubt, upon the report and recommendation of Whiting (1929), who stated that pans of this material "combine the qualities of durability and cleanliness; they are nearly stain-proof; they require no greasing," etc.

The Research Fellow's experience with this pan, however, raises serious doubt as to whether it is actually the most suitable for the purpose. As stated in the preliminary report (Merritt, 1931) pans of this type were found to have certain troublesome features. The new pans required a long period of *breaking in*, and could not be used without an occasional light greasing. There was no evidence, however, of any serious reduction in loaf volume due to very light applications of grease, without which there was frequent sticking of loaves to the pan.

What was perhaps the most objectionable feature of the prescribed "spotless metal" pans was the degree of *breaking in* required before acceptable and consistent results could be obtained. Even after the pans were all apparently broken in, it was found that a few pans that had been used less than others invariably gave erratic results. On

several occasions these few pans gave loaf volumes 70 to 80 cc. in excess of volumes produced under comparable conditions by the pans that were in habitual use. Typical instances of this are shown in Figure 13.

During the past few years a large volume of wheat and flour quality studies have been reported from the several laboratories of the Associate Committee on Grain Research, National Research Council of Canada. The baking technique employed in these studies has been based in principle upon the Werner (1925) fixed method, but instead of the baking pans now specified in the A. A. C. C. basic procedure they have used, and prefer to use, low sided tin pans. Dr. R. K. Larmour [8] states that the low formed tins are satisfactory in every way and require no greasing. The use of the low tins has, therefore, been deemed well worthy of study by the Fellow.

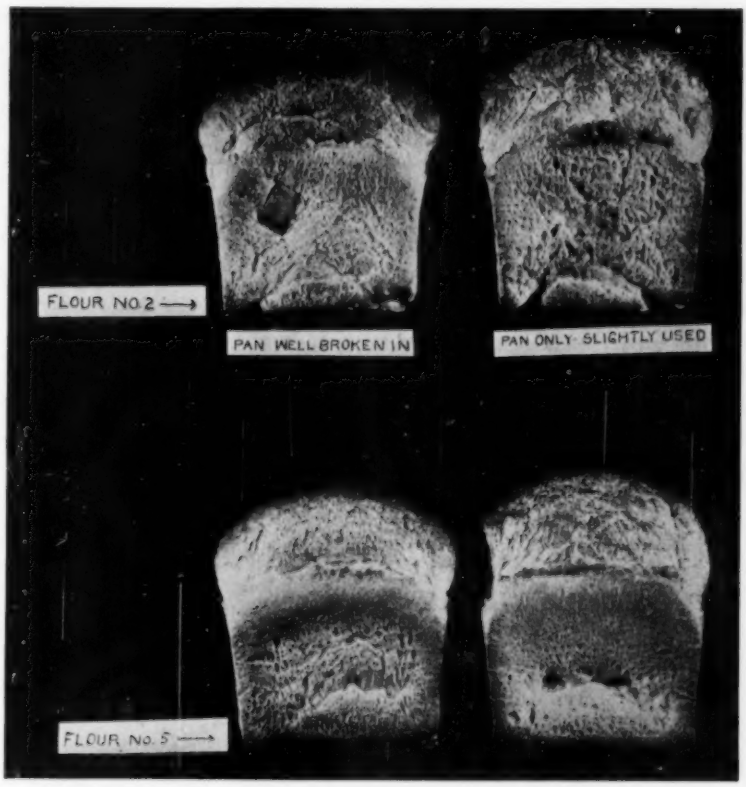

Fig. 13. Influence of "breaking in" spotless metal pans on external loaf characteristics.

The pan studies here reported involved 3 kinds of baking pans: (1) The prescribed spotless metal type; (2) pans of the same type and

[8] Personal communication.

dimensions, but made of heavy tin; and, (3) the low form tins, which are simply miniature pans of the ordinary commercial type. These are hereinafter designated, respectively, as the "old type," "high tins," and "low tins." The inside dimensions of the low tins were: Top length, 12 cm.; top width, 7.5 cm.; bottom length, 9 cm.; bottom width, 5 cm.; depth, 5 cm.

In the first series of tests 4 flours were used, and each flour was baked 30 times in each type of pan, with results as shown in Table XIII.

TABLE XIII

VOLUME AND VARIABILITY OF BAKES USING 3 TYPES OF PANS

Flour number	Number of individuals by each pan	Loaf volume using—		
		Old type	High tins	Low tins
		cc.	cc.	cc.
1	30	467	485	509
2	30	512	526	559
5	30	588	606	652
6	30	607	623	679
Average coefficient of variation		1.89	2.04	1.67

As is shown in Table XIII, the low tins always give larger loaf volumes than either of the higher sided, narrower pans. The old type pans gave the lowest loaf volumes, with the high tins intermediate. A slight advantage in variability is also in favor of the low tins, but the significance of these figures may be somewhat questionable.

In order to arrive at a better notion of the coefficient of variation using the low tins, flour No. 6 was re-baked 90 times, or 30 loaves per day for 3 consecutive days with results shown in Table XIV.

TABLE XIV

VARIATION OF TEST BAKES IN LOW TINS

Date baked	Number of individuals	Mean loaf volume	Coefficient of variation
		cc.	cc.
8/26/31	30	663	1.94
8/27/31	30	660	1.31
8/28/31	30	655	2.26
Average			1.84

A similar study using the old type pans is recorded in Table XV.

A comparison of values in Tables XIV with those in Table XV shows variability again in favor of the low tins. Other features in favor of the low tins were as follows: They required the least greasing

TABLE XV

VARIATION OF TEST BAKES IN OLD TYPE PANS

Date baked	Number of individuals	Mean loaf volume	Coefficient of variation
		cc.	cc.
8/31/31	30	595	2.33
9/1/31	30	579	2.71
9/2/31	30	581	2.16
Average			2.40

of any pans in the writer's experience; no special *breaking in* process was necessary, the pans giving the same results at the start as they did after considerable use; they are convenient to handle; and it is easier to introduce the molded dough into them than in the high sided pans.

The high tins appear to require some greasing, at least at the start, but whether they would need as much or as frequent greasing as the spotless metal pans is doubtful. No systematic study of this point was made. At any rate, they did not seem to require any special *breaking in*, and in this respect are superior to the spotless metal pans. They also give consistently larger loaf volumes than do the prescribed spotless metal pans.

In comparing the low forms with the high sided pans there at once arises the question as to which pan is superior from the standpoint of differentiating among the baking characteristics of various flours. Will the low tins give as informative baking results as the high pans?

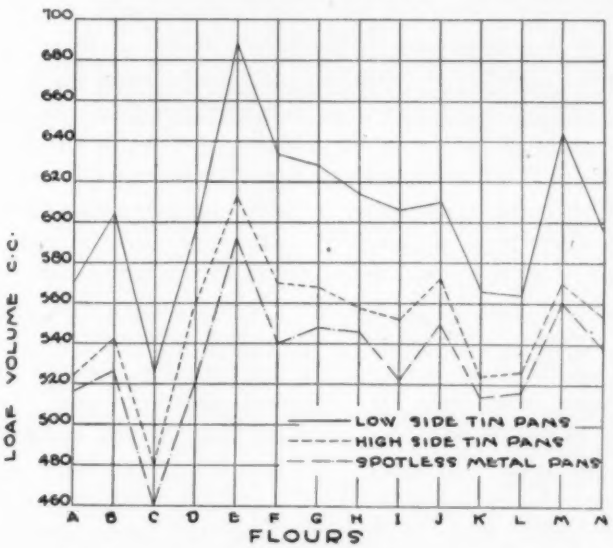

Fig. 14. Relation of pan type to loaf volume with 14 flours.

Figures 14 and 15, respectively, in which each point on the horizontal axis denotes an individual flour sample, show that the volume differentials among these miscellaneous flours are in substantially the same order regardless of the type of pan used. The actual volumes, however, run consistently highest for the low tins, and lowest for the old type pans, with the high tins intermediate.

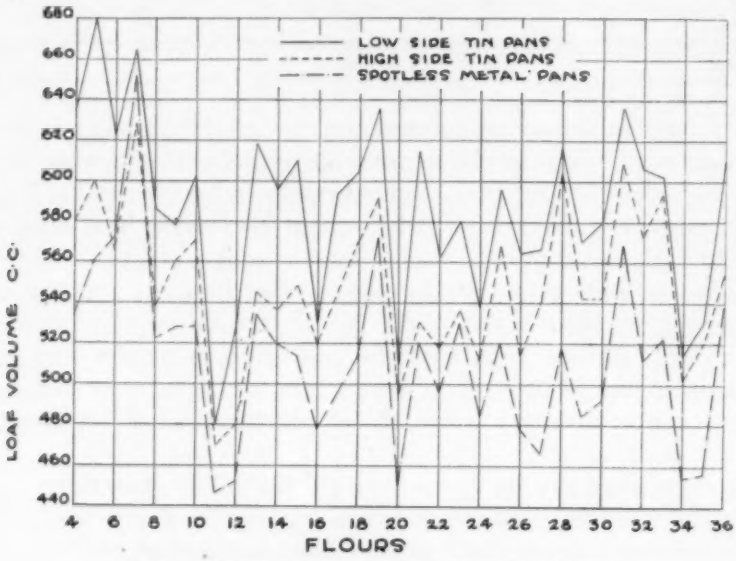

Fig. 15. Relation of pan type to loaf volume with 32 flours.

As to the suitability of the low tins in the matter of differentiation among flour characteristics other than volume, attention is directed to Figures 16 and 17. Loaves 1 and 2 were baked in the regulation old pans, and loaves 3 and 4 in the low tins from the same flour; numbers 5 and 6 show the internal loaf properties of numbers 1 and 2, while numbers 7 and 8 show the internal characteristics of numbers 3 and 4. The same differences in loaf characteristics are apparent regardless of the type of pan used. In every case the loaf on the left shows slightly more symptoms of *age* than the loaf on the right, both in external and internal properties. Loaf number 4 in Figure 17, is decidedly representative of the type of loaf produced by the low tins.

Various *differential* tests that are frequently used by flour technologists were tried, using the different types of pans. In all cases the loaves baked in the low tin pans showed the same differential tendencies as in the high pans. Typical results of experiments of this character are shown in Figures 18, 19, and 20, in which supplementary method B (differentials in fermentation time) was used with the 3 types of pans and with 3 flours. Plotting loaf volumes against time

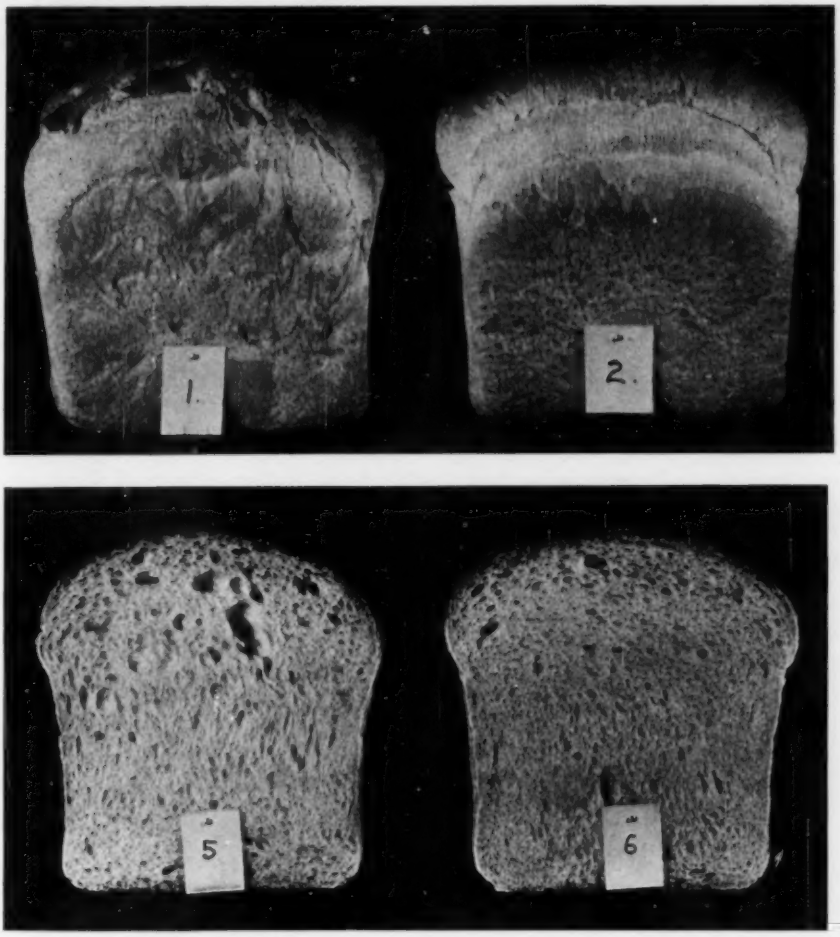

Fig. 16. External and internal characteristics of bread baked in old pans. Compare with Figure 17 where the loaves exhibited were baked in low form tins.

resulted in graphs of essentially the same curve and conformity regardless of the type of pan used. Bromate differential tests gave results of a similar character.

There was one property of the experimental loaves, however, with respect to which flours seemed to show less differentiation when baked in the low pans than when the high ones were used. That was the nature of the break on the loaf, due to oven spring. The loaves baked in the low tins quite consistently showed a smoother development or shred than the high pan loaves, many of the latter showing comparatively rougher or more ragged developments. Variations among flours with respect to the kind of break on the loaf was greater with the high than with the low pans.

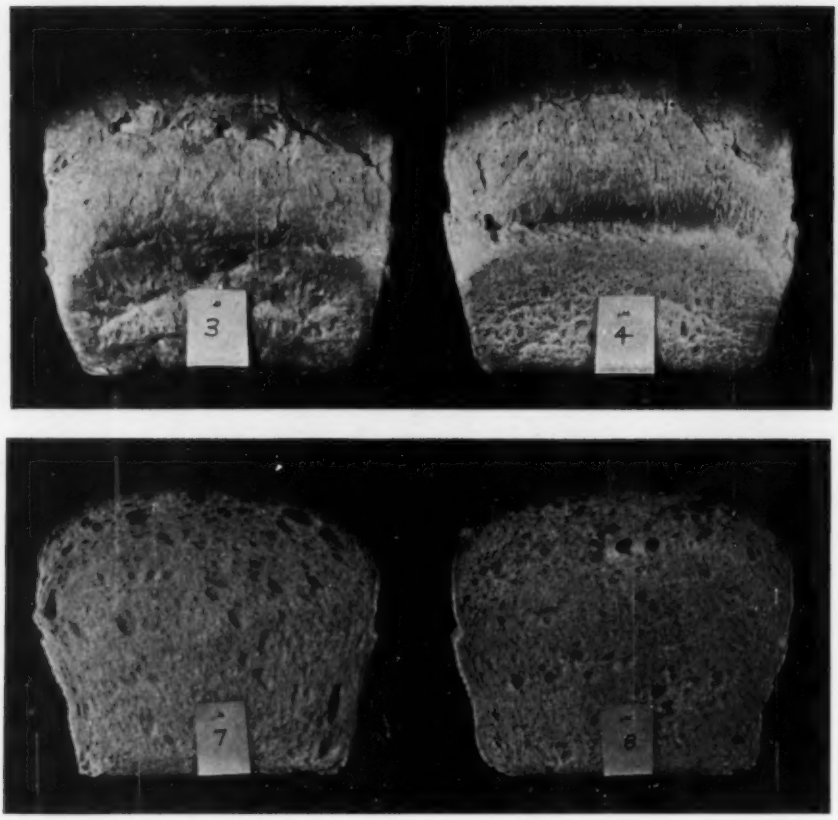

Fig. 17. External and internal characteristics of bread baked in low form pans. Compare with Figure 16 where the loaves exhibited were baked in the old pans.

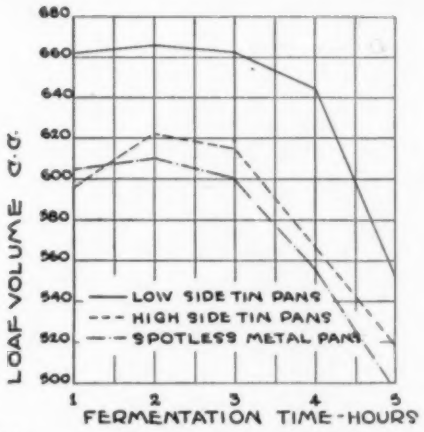

Fig. 18. Relation of fermentation time to loaf volume with flour No. 6—pan type variable.

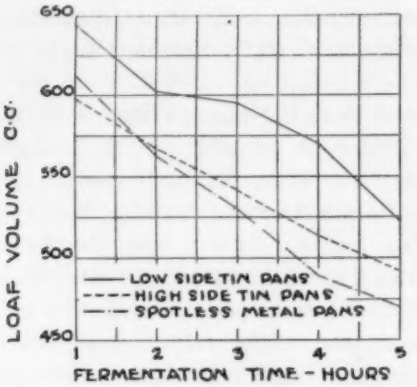

Fig. 19. Relation of fermentation time to loaf volume with flour No. 2—pan type variable.

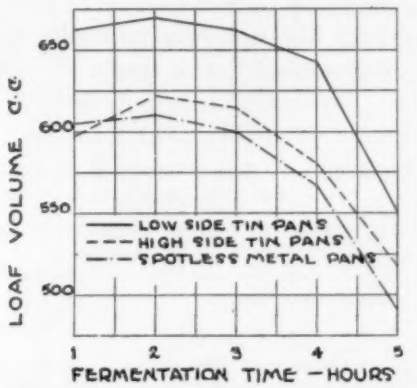

Fig. 20. Relation of fermentation time to loaf volume with flour No. 5—pan type variable.

Baking Tests Involving the Complete Elimination of Sugar from Baking Formula

The possibility of baking bread with the elimination of sugar from the formula is well known to all, and the procedure has often been used both in commercial and in laboratory test baking. In this type of baking, yeast leavening obviously depends upon the sugars originally present in the flour, together with sugars produced during fermentation by the flour diastases. In straight dough baking, using only simple ingredients, fermentation tolerance (the time range over which active yeast fermentation may be maintained) is predominantly dependent upon the maintenance of an adequate sugar supply in the dough. It follows then, that the elimination of sugar from the baking formula will necessitate a reduction in fermentation time; exceptions to this rule being found only among flours of abnormally high diastatic power.

A few preliminary trials by the Research Fellow indicated a very strong resemblance between test loaves baked without sugar and with

only 1½ hours of fermentation, and loaves from the same flours baked by the basic procedure with 2½% of added sugar and a fermentation time of 3 hours.

It was considered that the elimination of an ingredient together with a saving of 1½ hours in making the test is decidedly worthwhile, providing, of course, that other valuable features of the test are not thereby seriously minimized or destroyed. A more or less systematic study of possibilities in this direction was, therefore, undertaken.

Table XVI shows loaf volumes when 4 flours were baked without sugar and with fermentation periods reduced to 1½ and 2 hours, respectively, as compared with loaf volumes when the same flours were baked by the regular basic procedure. The 1½ hour fermentation doughs were punched once at the end of an hour; the 2 hour fermentation doughs received a first punch at the end of 1¼ hours, and a second punch ½ hour later. All were machine molded. Both the regulation and the low tin pans were used in this study.

TABLE XVI

LOAF VOLUMES AS AFFECTED BY ELIMINATION OF SUGAR AND REDUCTION OF FERMENTATION TIME

Flour number	Loaf volumes [1]					
	Regulation pans			Low tin pans		
	Basic procedure	No sugar 1½ hours fermentation	No sugar 2 hours fermentation	Basic procedure	No sugar 1½ hours fermentation	No sugar 2 hours fermentation
	cc.	cc.	cc.	cc.	cc.	cc.
1	467	526	471	509	547	534
2	512	562	596	559	629	619
5	588	610	662	652	672	711
6	607	613	517	679	652	587

[1] Each value is average of 6 individual bakes.

It will be noted from the values in Table XVI that the loaf volumes for the 1½ hour fermentation doughs without sugar, although slightly greater than the volumes for the regular basic procedure, were of substantially the same order of magnitude. The corresponding loaves of the two series resembled each other in all important features, including crust color differentials. In both series the loaf from flour No. 6 was palest of the four, indicating that it was on the verge of sugar exhaustion, and that it had the lowest diastatic power of the group. Actual diastatic value determinations proved this to be the case. This is further substantiated by the series having no sugar but a 2

hour instead of a 2½ hour fermentation period. Here the loaf volume of No. 6 dropped nearly 100 cc., while the volumes of the other 3 flours suffered no such loss, Nos. 2 and 5 actually showing an increase in volume. No. 6 had distinctly the lightest crust color of the four.

These tests suggest that when sugar is omitted from the formula, with other factors unchanged, a 1½ hour fermentation period will give baking results very closely approximating those obtained by the regular basic procedure.

Thirty-three miscellaneous baker's flours were then baked by both the regular basic procedure and by the 1½ hour fermentation procedure. All were punched and molded with the S-rolls.

Figure 21 shows graphically the comparative loaf volumes produced by the two procedures for each flour. The graphs show that in almost every instance the 1½ hour fermentation procedure with no sugar gave a greater loaf volume than the basic procedure, and in some instances the difference was as much as 60 cc. The two graphs have essentially the same conformity, however, showing that the two procedures give for all practical purposes the same volume differentials when applied to a miscellaneous group of flours.

Differentials in other important loaf characteristics, including smoothness, crumb structure, and crust color, were for all practical purposes the same in both series.

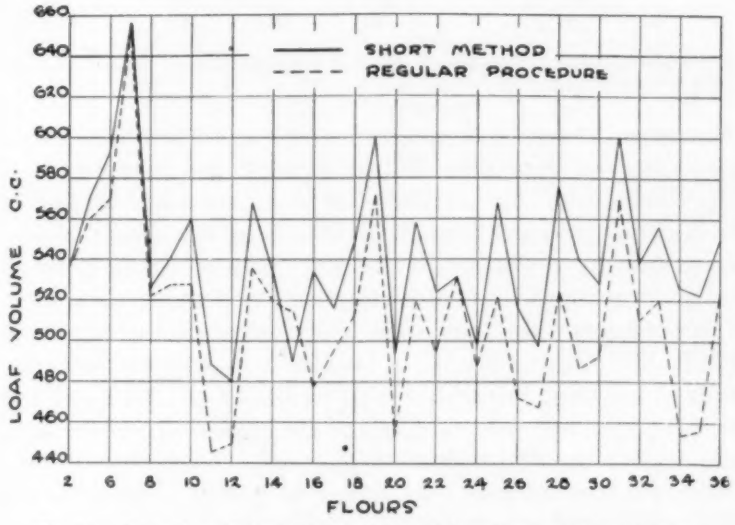

Fig. 21. Comparison of short method with regular basic procedure on 32 flours.

These and similar experiments, all of which need not be reported here, strongly suggest that tests without sugar and with a 1½ hour

fermentation period (hereinafter referred to as the "short method") can be made as informative as tests performed by the standard basic procedure. It is maintained by some, however, that the basic method alone does not give results that are sufficiently informative, and that one or more of the supplementary procedures must be used. This is sometimes, but not necessarily always, true. Nevertheless, it was deemed advisable to test the response of flours baked by the short method with certain supplementary procedures, especially supplementary procedures C (the bromate differential test) and D (tolerance toward mechanical mixing).

Experiments reported in Table XVII show that the short method will indicate a flour's response to additional mixing in much the same manner as when the flours are baked by the standard basic procedure. The Hobart-Swanson mixer was used.

TABLE XVII

SUPPLEMENTARY METHOD D AS APPLIED TO THE SHORT BAKING METHOD

	Loaf volumes [1]			
Flour number	Mixed for 1 minute	Mixed for 2 minutes	Mixed for 2½ minutes	Mixed for 3 minutes
	cc.	cc.	cc.	cc.
1	534	599	594	617
2	619	668	679	667
5	711	698	680	688
6	587	736	759	788

[1] In the first 3 columns each figure is an average of 6 individual tests. Figures in last column are averages of duplicates.

When the bromate differential test was applied to flours baked by the short method, it became at once apparent that tolerance to $KBrO_3$ is quite different by that method than by the regular basic procedure. With the short method more $KBrO_3$ is required to produce a given response than by the standard method. The tolerance is far greater by the short method, and correspondingly greater amounts are necessary to *break down* the flour than when baked by regular procedure.

Table XVIII gives results of studies with 4 flours, showing their bromate tolerances, respectively, when baked by the short method. These were bleached baker's flours of the type that frequently, if not usually, show a negative volume stimulation when baked by the specified standard method plus 1 or 2 mgs. of $KBrO_3$. All were machine molded, and baked in low tins.

The data in Table XVIII show tolerances and lack of sensitiveness to varying increments of $KBrO_3$ that are surprising to one who is familiar with what happens when a similar differential treatment is

TABLE XVIII

BROMATE TOLERANCES USING SHORT BAKING PROCEDURE

$KBrO_3$ per 100 gms. flour	Loaf volumes			
	Flour 1	Flour 2	Flour 5	Flour 6
mgs.	cc.	cc.	cc.	cc.
0	548	635	637	651
1	558	615	674	680
2	525	625	685	712
3	542	612	686	743
4	497	610	647	708
5	498	593	660	709
6	480	566	659	670
8		552		641
10		523		566
12		495		529

applied under the regular procedure involving the 3-hour fermentation period and hand molding. A portion of this tolerance is doubtless due to the situation discussed in connection with Table XI, and Figures 6, 7, and 8, where it was shown that tolerances toward bromate are greater with machine than with hand molding. That much of the extra tolerance, which is in a large measure due to the shortening of the fermentation period, is shown by data in Table XI. This flour was baked both by the short and the regular methods, using varying amounts of $KBrO_3$. Molding was done with the S-rolls.

TABLE XIX

BROMATE TOLERANCE OF UNBLEACHED CLEAR FLOUR BY SHORT VERSUS REGULAR BAKING PROCEDURE

$KBrO_3$ per 100 gms. flour	Loaf volumes	
	Short baking method	Regular baking procedure
mgs.	cc.	cc.
0	550	450
2	592	615
4	635	627
6	665	557
8	662	
10	660	
12	655	
14	657	
16	620	
18	625	
20	610	

Data in Table XIX indicate that in so far as loaf volume is concerned, 6 mgs. of $KBrO_3$ caused more *break down* when the standard regulation procedure was used than did 20 mgs. when the baking was

done by the short method. By the latter method, furthermore, it was possible to vary the amount of $KBrO_3$ from 4 to 20 mgs. without affecting any pronounced changes in loaf volume, and development of the so-called *age* symptoms in the crumb did not become apparent until 10 mgs. had been used. These symptoms increased but very slowly by the use of from 10 to 20 mgs.

It would appear from these findings that the short method is not well suited for studies of tolerance toward oxidizing agents, at any rate this seems to be the case in so far as potassium bromate is concerned. Upon further inquiry into this situation, however, it was found that *potassium iodate* may be substituted for the bromate with highly satisfactory results.

Figure 22 shows photographically the manner in which the baking characteristics of the unbleached clear (the same as used in the preceding experiment) are affected by exceedingly small increments of KIO_3 when using the short method of baking. The loaves were molded by the S-rolls. It may be recalled that the use of the S-rolls tended to minimize the age symptoms produced by over-oxidation with potassium bromate (see Figure 8). In the experiments illustrated by Figure 22, however, and notwithstanding the fact that both the short method and the S-rolls were employed, it is obvious that the effect of KIO_3 is most critical and drastic in very small quantities.

Nine miscellaneous baker's flours, selected at random from a large group of samples, baked by the short method with 1 mg. KIO_3 and molded by the S-rolls, gave results shown in Table XX.

TABLE XX

EFFECT OF 1 MG. KIO_3 ON LOAF VOLUMES OF 9 BAKER'S FLOURS USING SHORT BAKING PROCEDURE

Flour number	Loaf volumes	
	Untreated	1 mg. KIO_3
	cc.	cc.
9	540	492
10	560	557
13	570	515
16	537	490
19	587	517
21	557	487
31	600	497
35	522	490
36	555	475

These data show that there was a loaf volume reduction caused by 1 mg. KIO_3 in all cases. It will be noted from Figure 22 that even with a freshly milled unbleached clear, maximum loaf volume stimula-

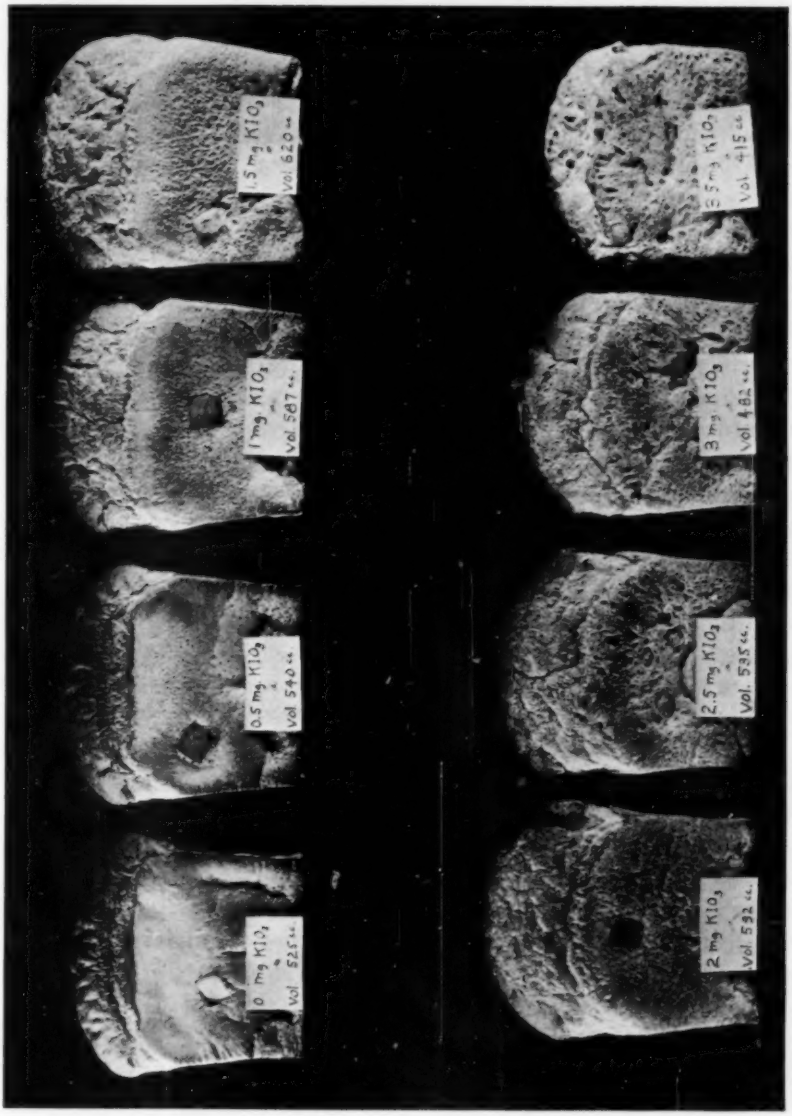

Fig. 22. Showing manner in which baking characteristics of an unbleached clear flour was affected by small increments of KIO_3 when using the short method.

tion occurred with as little as 1.5 mg. KIO_3, and that the effect of 3 mgs. of KIO_3 was practically disastrous. These experiments strongly suggest that when using KIO_3 it may be appropriate to reduce the increments from 1 mg. (as is customary for $KBrO_3$) to 0.5 mg. per 100 gms. flour.

With this modification of procedure in the case of supplementary procedure C, it is not overstating the case to say that this short method, involving the elimination of sugar and a reduction of $1\frac{1}{2}$ hours of fermentation time, can be made to serve many, if not nearly all, ordinary purposes for which experimental baking tests are conducted.

Miscellaneous Experiments

There are a number of manipulative details in laboratory test baking that are generally considered to be more or less trivial as compared with factors such as mixing, molding, fermentation time, etc. Nevertheless, it is a matter of commonplace knowledge that in any testing procedure involving a multiplicity of *biological factors* some of these supposedly trivial details occasionally assume an importance far beyond expectations. During the work of the Research Fellow certain matters having potentialities of this general character have occasionally suggested themselves, or have been suggested by others as subjects worthy of experimental consideration.

In most of these cases the results were reasonably definite to such a degree that beyond a few brief trials no extensive or prolonged investigations were deemed necessary.

Studies on Yeast Handling

Experience with yeast at all times confirmed the findings of Herman and Hart (1927) who found no evidence of deterioration of yeast during several days of storage. Almost any lot of yeast could be used over a period of several days without significant change in baking properties.

When enough yeast was crumbled up to last through a day's run, the only precaution that seemed necessary was to keep it covered to avoid loss of water by evaporation. In one experiment 30 loaves were baked with yeast standing all day at room temperature, while another 30 were baked with yeast kept constantly in the refrigerator at 11° C. except while weighing out the individual portions. The first lot averaged 576 cc. in volume, and the second lot 578 cc., with no differences whatsoever in loaf characteristics.

Yeast suspensions in water were found to give the same results as ordinary yeast, throughout an entire day's run, regardless of whether the suspension was kept in the refrigerator at 11° C. or in the fermen-

tation cabinet at 30° C. It is necessary, of course, when using a yeast-water suspension, to stir vigorously during the withdrawal of each portion with the pipette. In these experiments 120 gms. of yeast were suspended in 390 cc. of water with a malted milk stirrer. This makes 500 cc. of suspension; 25 cc. of which contain 6 gms. of yeast and 19.5 cc. of water.

Baking Pan Temperature Studies. A few experiments were made for the purpose of noting the effect upon loaf characteristics when the temperature of the pans varies considerably from that of the dough at panning time. In each series half the pans were kept in the refrigerator, and half on a radiator where they reached a temperature of about 50° C. The results are noted in Table XXI.

TABLE XXI

EFFECT OF BAKING PAN TEMPERATURE ON LOAF VOLUMES

	Flour 2	Flour 5	Flour 6
Number of individuals in each experiment	30	30	30
Mean loaf volume with pans at 11° C. (cc.)	504	557	566
Mean loaf volume with pans at 50° C. (cc.)	532	588	556
Coefficient of variation (pans at 11° C.)	1.18	2.44	1.48
Coefficient of variation (pans at 50° C.)	1.96	2.70	2.10

Here it is shown that with flours 2 and 5 panning in pans about 20° C. warmer than dough temperature gave larger loaf volumes than panning in pans approximately 20° C. colder than dough temperature. The reverse was true with flour 6. Flours 2 and 5 are high diastatic flours while flour No. 6 is low in diastatic power, which may possibly explain the different reactions of the flours to the same differentials in pan temperatures. These are extreme conditions, of course, and it is doubtful if the effect of the differential between average laboratory room temperatures and 30° C. is ordinarily large enough to necessitate the precaution of bringing the pans to 30° C. before panning the dough. The experiments are of interest, however, in again demonstrating variations in the responses of individual flours to the same differentials of treatment.

Baking Without Top Heat in Oven

In the previous report of the Research Fellow's activities (1931) brief mention was made of the chance that there might be certain advantages in eliminating all top oven heat in experimental baking, the chief possibility being that the elimination of top heat might afford a better differentiation as to the top crust characteristics among test loaves from different flours. This idea was experimented with using oven Y with both top and bottom heat on "high"; oven Y with the top heat turned completely off; and oven Z (the small 2-loaf oven)

which has no provision for top heat at all. The studies involved baking different flours, and baking the same flour with different fermentation periods, for the purpose of establishing systematic variations in loaf characteristics.

The tests showed characteristic differences among loaves baked under the different heat conditions even though there was satisfactory compliance with the present specifications regarding maintenance of degree and uniformity of temperature with the thermometer set in the prescribed location. There were no pronounced differences in loaf volume, *crust color* being the property most noticeably affected. This confirmed the findings of the previous report (1931).

Loaves baked without top heat, whether in oven Y or in oven Z, were not only lighter in crust color (other factors being equal), but they permitted better crust color *differentiation* than did loaves baked with both top and bottom heat. Blish, Sandstedt, and Platenius (1929) have pointed out the importance of crust color as a diagnostic symptom of diastatic activity, or "gassing power"—and therefore of fermentation tolerance—in flours. Flours 2 and 5 were both fairly high diastatic flours, with 2 considerably the higher of the two. Their crust colors could not be differentiated when baked with both top and bottom heat in oven Y, but when baked without top heat a difference was quite apparent with flour 2 showing a noticeably darker crust color than flour 5, as is in accord with their respective diastatic values.

It was also noted that even in duplicate bakes of the same flour, if one loaf happened to rise slightly higher in the oven, with both top and bottom heat, the higher loaf invariably showed a darker top crust color. This tendency was much less pronounced when top heat was eliminated.

It was not possible to adequately record slight crust color differences numerically by any means in possession of the writers, and, therefore, no numerical data are offered in connection with these experiments dealing with crust color differentials. There are doubtless many technologists who will feel that the importance of crust color is here overemphasized. Nevertheless, it is a symptom that can be made to serve a most useful purpose, and because of the findings here recorded, the writers are inclined to favor the elimination of the top oven heat in experimental baking.

The turning off of the top heat in oven Y apparently did not seriously interfere with the satisfactory oven performance. It decreased the frequency of the make and break of the electrical current by one-half, causing the temperature to drop more rapidly and rise much more slowly than when both top and bottom heat were on. Temperature curves are shown in Figure 23.

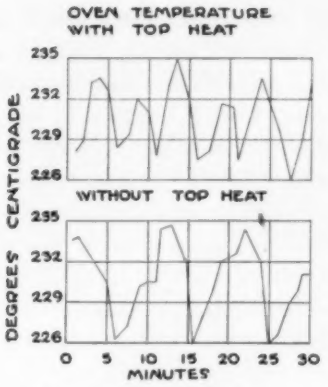

Fig. 23. Temperature relations in oven with and without top heaters in operation.

Molder Adjustment Studies

It was stated in the previous report (1931) that in operating the Thomson Roll Molder the "adjustment of the compression plate was found to be far more critical than the setting of the sheeting rolls," and that these observations were in complete disagreement with the findings of Geddes, et al. (1931), who reported that "variations in the setting of the sheeting rolls were found to have a more pronounced effect on volume and texture than variations in the adjustment of the compression or former plate."

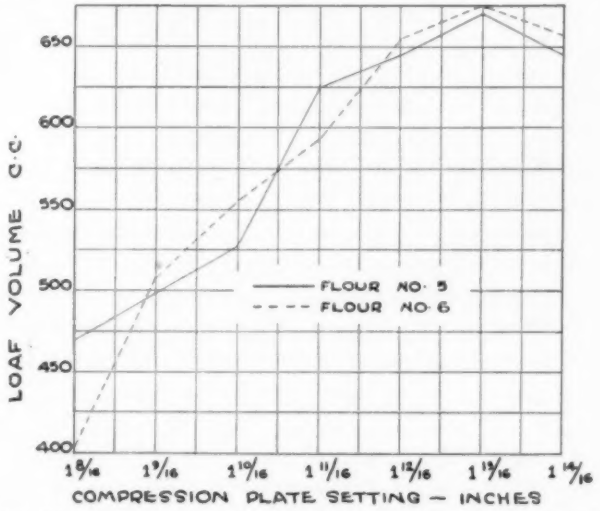

Fig. 24. Relation between Thomson Roll Molder adjustments and loaf volume; compression plate adjustments.

In view of the strikingly contradictory nature of these results from two laboratories supposedly using the same type of machine, it was

felt that a repetition of these experiments was advisable. The results of these experiments, however, merely verified the Research Fellow's previous findings (1931), as is shown in Figures 24 and 25. No explanation of the disagreement with Geddes et al. (1931) suggests itself, unless there could have been some obscure differences in the construction and performance of the two machines.

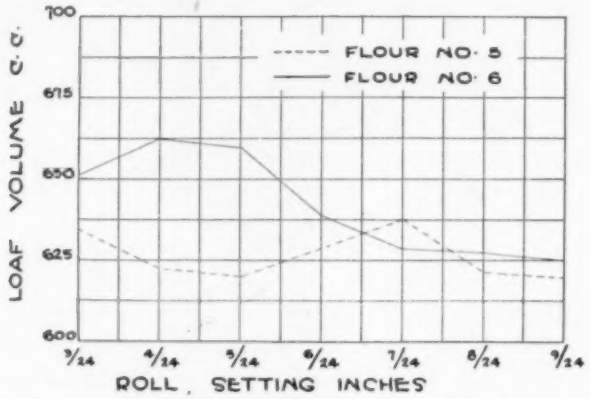

Fig. 25. Relation between Thomson Roll Molder adjustments and loaf volume. Setting of sheeting rolls.

Salt Tolerance Study

A brief inquiry as to the manner in which a flour may be expected to respond to sodium chloride differentials was made, using two flours, the low tins, and the short baking method without sugar. The effects of these salt differentials on loaf volume are shown in Figure 26.

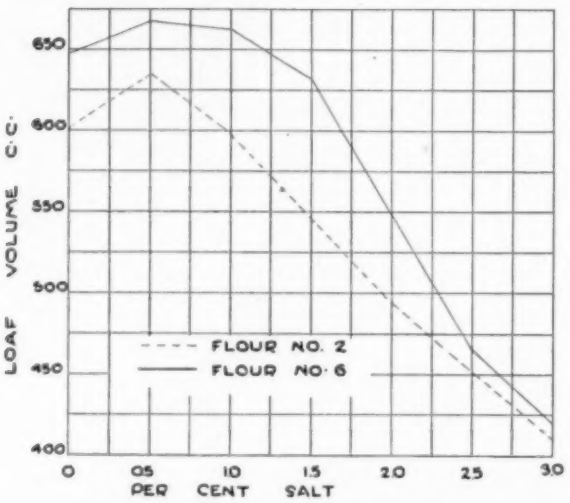

Fig. 26. Relation of salt concentration to loaf volume.

Increasing the salt concentration above the prescribed 1% caused proportional reductions in loaf volume with both flours. This is in agreement with Herman and Hart (1927).

Response of Miscellaneous Flours to Various Modifications of Baking Technique

Considered in the aggregate, the experiments of the Research Fellow show certain definite tendencies as to the manner in which test loaf characteristics are influenced by various modifications of technique and environment. Attention has been directed to the fact that not all flours (even of supposedly the same grade and class) respond equally to almost any modification or differential of treatment that might be selected.

To what extent do flours actually vary in their response to some of the numerous "differential treatments" or "supplementary procedures" that may conceivably be used in laboratory flour testing? Although time and facilities did not permit any exhaustive researches in this field, an effort was made to survey certain phases of the situation, and it is believed that the data obtained are at least suggestive.

For the purposes of these experiments flour samples were solicited from a number of mills in various parts of the United States and Canada. More than 30 of these mills generously donated the material in the amounts requested. The series included flours from southwestern, northwestern, eastern and Pacific Coast mills of the United States, as well as Canadian mills. The flours are here indicated by number, and their analyses, respectively, are shown in Table XXII.

Based on respective protein contents, alone, most of the flours in Table XXII would be classed as "strong" flours. It will be noted that in the great majority of instances the absorption is substantially higher than the prescribed *normal* of 58% on a 15% moisture basis.

The results of this series of studies are shown in Figures 5, 14, 15, 21, and 27 to 31 inclusive. Loaf volume measurements are plotted vertically and the flour sample numbers horizontally. Each chart shows 2 or more graphs, and represents a "differential" of treatment. The degree of similarity between the graphs on any individual chart indicates the uniformity with which the various flours responded to the particular *differential* under consideration. This was considered to be the simplest and briefest method in which to present data showing both manner and the degree of response by a miscellaneous group of flours to various differential treatments. No attempt has been made to give detailed consideration to bread characteristics other than *loaf volumes* in reporting these series of *differential* studies. To undertake to thoroughly report and discuss all other bread properties

TABLE XXII

CHEMICAL ANALYSES OF FLOURS STUDIED IN THE TESTS

Flour number	Protein	Ash	Moisture	Estimated absorption [1]
	P.ct.	P.ct.	P.ct.	P.ct.
2	10.9	0.43	13.4	58
5	12.3	.47	13.9	58
6	15.1	.47	11.2	65
7	14.4	.42	11.4	64
8	11.9	.41	11.3	62
9	13.3	.45	12.1	63
10	13.9	.48	11.7	61
11	12.2	.62	13.7	58
12	12.1	.41	12.2	61
13	12.2	.44	11.3	62
14	13.7	.46	11.5	60
15	12.5	.40	12.0	60
16	12.5	.45	12.3	61
17	11.7	.42	11.9	57
18	12.2	.45	12.4	61
19	13.8	.43	12.6	61
20	12.0	.46	12.5	61
21	11.5	.41	12.5	61
22	11.7	.47	12.6	60
23	11.5	.41	12.8	62
24	10.7	.52	12.3	62
25	12.3	.43	13.1	61
26	11.3	.43	12.2	60
27	11.9	.40	12.9	62
28	11.7	.44	12.8	62
29	11.1	.48	12.9	62
30	11.8	.47	12.8	62
31	12.3	.45	12.6	61
32	12.4	.42	12.5	61
33	11.3	.45	12.7	61
34	11.7	.44	12.7	61
35	11.6	.47	12.7	61
36	12.2	.45	12.9	62

[1] On 15% moisture basis.

would involve far more time, labor, and journal space than the situation demands, and would perhaps in no way alter the main conclusions to be drawn from the experiments.

Four of these Figures have already been presented and discussed earlier in the report. They include Figure 5, showing effects of molding with the S-rolls as compared with the Thomson Roll Molder; Figures 14 and 15, showing comparative results with different types of baking pans; and Figure 21, which shows comparative loaf volumes obtained by the regular basic procedure versus the short method in which sugar is eliminated and the fermentation time reduced from 3 to 1½ hours.

Effect of Elimination of Top Oven Heat. Chart 16 shows that the presence or absence of top heat in oven Y was of no very serious consequence as affecting loaf volume differentials among a group of

flours. There is a decided similarity between the two graphs. With some flours the absence of top heat gives slightly larger volumes, while with others the opposite was true, but the graphs show the same general conformity. The matter of crust color differentials in this series has been discussed elsewhere.

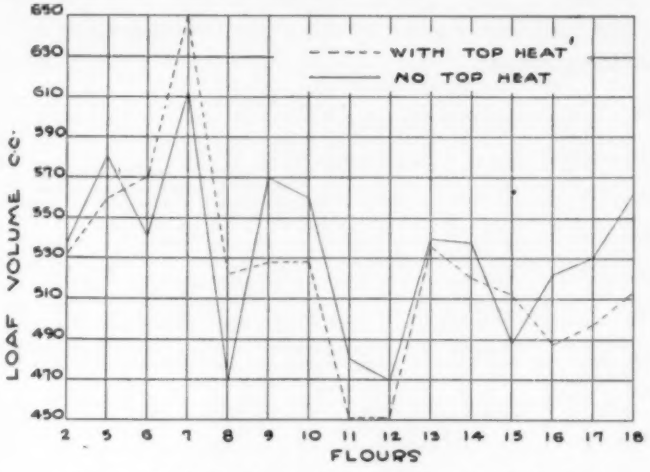

Fig. 27. Top heat and no top heat in relation to loaf volume.

Sugar Differential Tests. It is now generally realized that low diastatic or "gassing" power in certain flours may be a decidedly limiting factor in straight dough baking. This has been recognized by many workers, beginning with Wood (1907) and has been more recently discussed by Sherwood and Bailey (1926), Blish and Sandstedt (1927), Fisher and Halton (1929), Moen (1930), Jorgensen (1931), Markley and Bailey (1931), Elion (1932), and others too numerous to mention. Experimentally milled flours are especially likely to be low in gassing power, and unless this is adjusted by the use of extra sugar, or by diastatic preparations such as malt, sprouted wheat, etc., or by a reduction in fermentation time, the regular basic procedure will not yield informative results.

The flours shown in Table XXII varied somewhat in diastatic or gassing power, as was indicated by crust color differences. The entire series was baked using 5 gms. instead of $2\frac{1}{2}$ gms. of sugar, as prescribed in the basic procedure, with differential results as shown in Figure 28. Figure 22, previously discussed, compares the basic procedure with the short method using no sugar, while Figure 29 compares the use of 5 gms. of sugar, with no sugar, but with the reduced fermentation period.

Figure 28 shows that, with the exception of flour No. 8, the use of

5 instead of $2\frac{1}{2}$ gms. of sugar increased the loaf volume in every instance. For certain flours, such as 6, 9, and 12, the volume increases, due to additional sugar, were far greater than for other flours. These flours were all in the *low diastatic* group, as might be expected. In general, the crust colors of loaves baked with 5 gms. of sugar were too dark to permit of any useful differentiation. For the most part, however, the graphs in Figure 28 show similar tendencies with respect to high and low points.

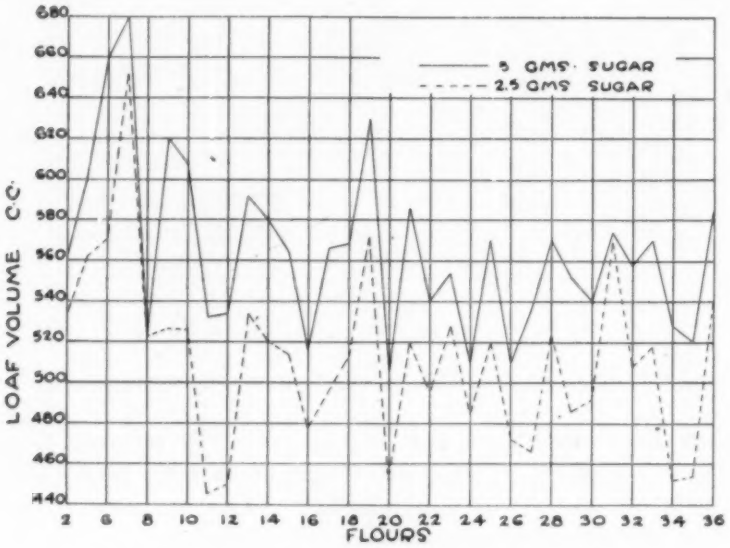

Fig. 28. Showing relation between sugar supply and loaf volume.

Figure 29 compares results using 5 gms. of sugar with those secured by the short method with sugar entirely left out of the formula. Here the loaf volumes are found to be in closer agreement than was found in Figure 28, where the use of 5 gms. of sugar was compared with $2\frac{1}{2}$ gms. The short method, without sugar, would be given the preference over the "5 gms. of sugar" method for this flour series, because it gave lighter crust colors that could be differentiated in contrast to the very dark crust colors produced in the series baked with 5 gms. of sugar. In a series of *experimentally milled flours*, however, it is highly probable that the use of 5 gms. of sugar would be preferable, if not absolutely essential, unless the low diastatic power of the experimentally milled flours is compensated for in some other way.

Use of Sprouted Wheat Flour. The use of sprouted wheat flour for supplementing low diastatic flours in the experimental baking tests is advocated by Markley and Bailey (1931), but they present no

evidence to show that this procedure offers any special advantages that are not secured by use of additional sugar. The latter is obviously a simpler procedure; furthermore, sugar is a distinct individual chem-

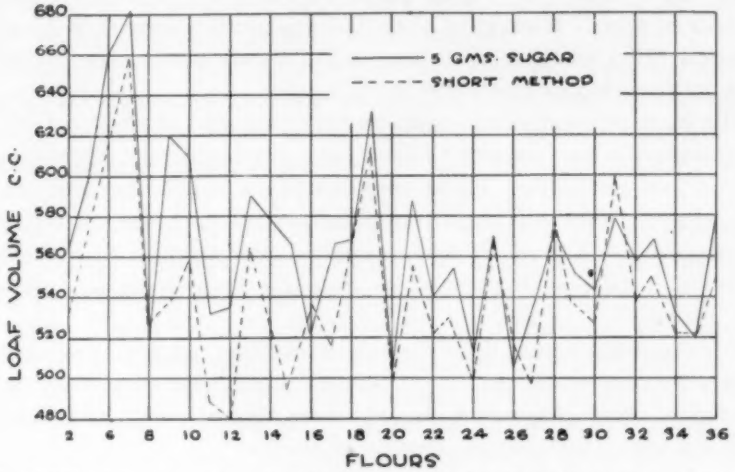

Fig. 29. Relation between sugar supply and loaf volume; short method and no sugar compared with basic procedure plus extra sugar.

ical substance obtainable in a high state of purity, whereas, sprouted wheat is a complex mixture of substances containing proteolytic as well as diastatic enzymes.

It was thought worthwhile to make a series of comparable tests supplementing the basic procedure with 2½ gms. of sugar, on the one hand, and 3% of sprouted wheat flour (made according to the pro-

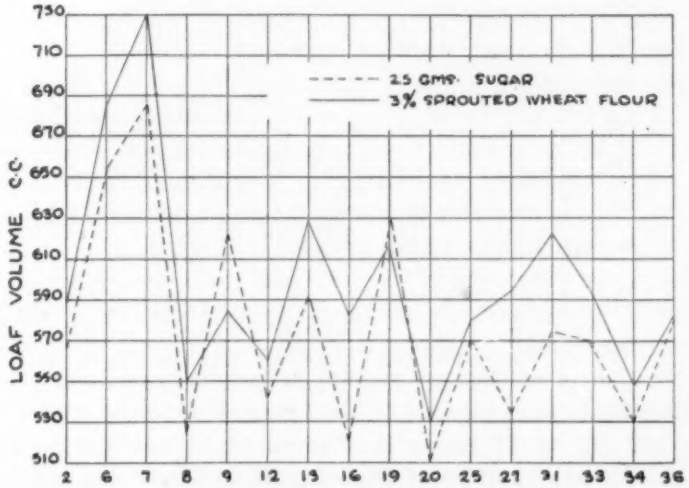

Fig. 30. Comparison of loaf volume results using 2.5 gms. sugar and 3% sprouted wheat flour.

cedure of Markley and Bailey), on the other. These tests involved 16 flours, and the results are shown graphically in Figure 30. With regard to loaf volume differentiation among the various flours, Figure 20 discloses no decided advantage favoring either method. The use of sprouted wheat flour gave slightly larger loaf volumes in 13 of the 16 flours, but the order of volume differentials among the individual flours was practically the same in each case.

The increased sugar series showed slightly darker crust colors than the sprouted wheat series. There were no important differences in internal loaf properties. The *sprouted wheat doughs* became slightly sticky during fermentation, although no actual *slackening* of the dough was observed. This stickiness, however, was enough to make them harder to handle with the S-rolls than the doughs of the increased sugar series.

The sprouted wheat flour series disclosed no particular advantages that the writers could detect, and based upon this somewhat limited experience their preference is, at least tentatively, given to the increased sugar technique, since it is simpler, more convenient, and offers advantages with regard to stickiness of doughs.

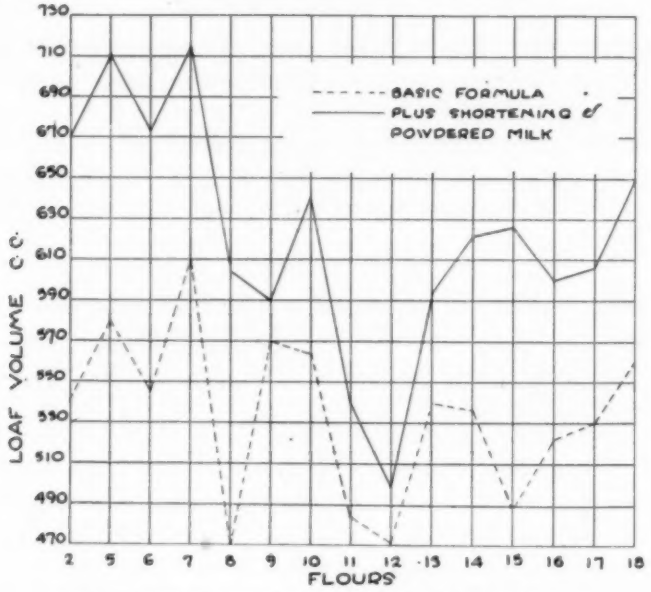

Fig. 31. Effect of shortening and powdered milk on loaf volume.

Tests with Shortening and Milk. Figure 31 shows loaf volume differentials among 15 flours as affected by superimposing 5 cc. of melted shortening (lard) and 5% of milk powder upon the regular basic procedure. The addition of these extra ingredients, commonly

used in modern commercial baking, increased the loaf volume for all flours, and in most instances the increase was a large one. Here again, however, it is apparent that, with few exceptions, the *order* of volume differentiation among the flours was essentially the same whether baked with or without the additional ingredients.

The milk sugar (not being fermentable by yeast) gave a dark crust color to all loaves, even those on the verge of diastatic exhaustion. This indicates that crust color cannot be usefully correlated with diastatic activity when milk is used as a dough ingredient.

General Discussion

The foregoing experiments suggest individual matters worthy of study and further investigation. Considering these studies in the aggregate, however, one is unavoidably impressed by the fact that regardless of the modification of treatment selected for use, the *relative differences* among various flours, as manifested by loaf volumes (and other important test loaf characteristics) were in essentially the same order. In case of strict necessity one could accustom himself to almost any of a large number of possible modifications of procedure. This means simply that in contemplating improvements in the present specifications for basic procedure, effort may properly be confined to items such as economy of time and equipment, convenience to the operator, and reduction of variability among different laboratories. These are the issues with which the conclusions, suggestions, and recommendations of the Research Fellow and his associates will be largely concerned.

The present A. A. C. C. Standard Experimental Baking Test provides for a "Basic Procedure" and four so-called "Additional and Supplementary" tests. In addition to the four that are specified, it should be perfectly obvious that many others could be suggested and used as occasion might require.

Surely it is as permissible to vary factors such as yeast, sugar, salt, etc., as it is to vary absorption, fermentation time, and degree of mixing. The essential thing is the establishment of a standard and uniform foundation, or *point of departure* for the subsequent tests or for modified procedures. Mention of "Additional and Supplementary" tests should either be deleted from the written specifications of the Standard Experimental Baking Test, or if this is not done, the number should be increased to include many others that are just as rational, informative, and permissible as are the four that are specified in the present Standard Experimental Baking Test.

In the interests of maximum convenience and simplicity, with a minimum of required time, operations, and ingredients in the basic

procedure, the writers are at present inclined to the view that the short method, involving the complete elimination of sugar, and a reduction of fermentation time from 3 to 1½ hours, is well worthy of serious consideration and further investigation. Although, at first glance, this may be regarded as a drastic change, and somewhat out of harmony with certain ideas with reference to *gluten stability*, there is nothing in the experience of the Research Fellow to show that it is not an entirely rational flour testing procedure. Its rationality is further strongly substantiated in some recent detailed investigations by Blish and Hughes (1932), who conclude, "The Standard A. A. C. C. Basic Procedure may well be regarded as primarily a *diastatic activity* test, with depth of crust color as perhaps the most conspicuous symptom of fermentation tolerance. Those test loaf properties that reflect the character of the *gas-retaining* agency, i.e., the gluten, have diagnostic value only when there is definite assurance that there has been an adequately maintained gas production." These ideas are strikingly illustrated in Figure 32. Flour 1 is an 11% protein flour of high diastatic power, while flour No. 2 is an extra strong northwestern flour containing nearly 15% protein but low in diastase. In each of the two tests, both flours were given the regulation 3-hour fermentation period. When no sugar was used flour No. 1 gave as large a loaf (nearly 600 cc.) as when the regulation 2.5 gms. of sugar were used, due to its diastatic power. The high protein flour (2A) gave a very small white loaf when subjected to a 3 hour fermentation period without sugar, but with 2.5 gms. of sugar it gave a loaf of more than 700 cc. volume.

Flour No. 2 had no chance to show its gluten properties until baked under conditions insuring adequate gas production. The gluten of flour 1 was inferior in quantity, if not in quality to that of flour 2, but flour 1 clearly has superior fermentation tolerance for it was able to support fermentation over the 3-hour period without any sugar, and by virtue of its diastatic power alone.

Moen (1930) stated that the baking formula "must have time and temperature so adjusted that diastatic capacity is severely taxed." He varied the fermentation time, and found the A. A. C. C. specified time to be the best suited for this purpose. Complete elimination of sugar, however, will permit a reduction of time without substantially changing the picture produced by the present basic procedure. Removal of sugar from the basic procedure formula simply enables flours to show the characteristic differences in fermentation tolerance with a minimum of fermentation time. Since this appears to be the primary purpose of the basic procedure, in the final analysis, and since the use of sugar simply extends the time to which doughs must be fermented

to show the same differences, why not use the short method as the basic procedure, with the understanding that all other methods are "supplementary" procedures? Why spend unnecessary effort in taking a time exposure if a snapshot will give the same picture?

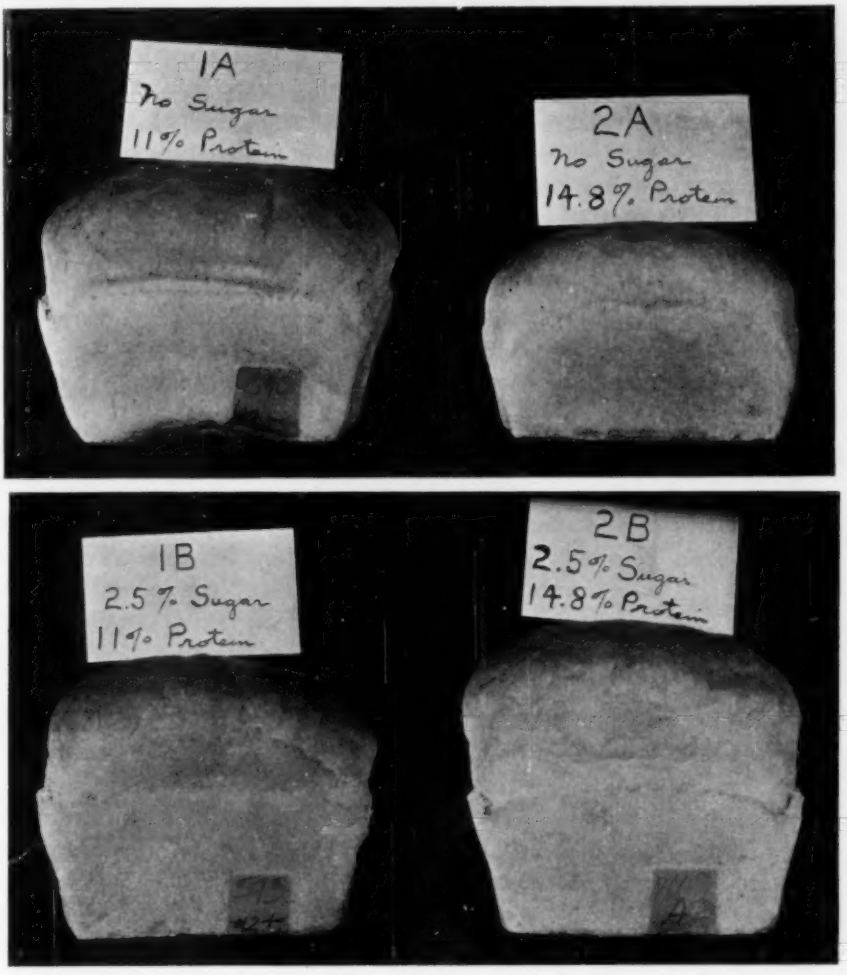

Fig. 32. Baking results with high diastatic flour compared to that of high protein flour of low diastatic value.

It is believed that a substantial reduction in variability among different laboratories can be accomplished through the use of S-rolls, or some mechanical contrivance of a similar character, in the operations of punching and molding the doughs. This, however, can be actually proved or disproved only by systematic collaborative testing among

different laboratories. It must be realized, moreover, that in collaborative testing of this type, only laboratories having the same oven facilities and precise fermentation temperature control can participate; otherwise the results are likely to be meaningless.

There is no reason why, with normal, average flours and proper equipment, a moderately experienced operator cannot perform a single test bake with a reasonable expectation of accuracy within ± 10 cc. in loaf volume.

For flours of exceptional potentiality for large loaf volume the probable error may exceed this figure, while with flours of less than normal capacity for producing large loaves the probable error should be less than 10 cc.

Variation caused by lack of yeast uniformity is not believed to have been a serious factor in these studies, although occasional inconsistencies occurred that could be accounted for in no other way.

Conclusions, Recommendations, and Suggestions

These are based upon observations and findings throughout the entire period of the Research Fellowship activities, and each item and phase of the test is dealt with individually. It is realized, of course, that certain of these recommendations could not be put into immediate practice even if officially adopted, since they involve mechanical contrivances that cannot yet be purchased on the open market. There are certain features concerning which definite recommendations at this time would be premature, but which are designated as well worthy of further study or verification.

There is perhaps one general recommendation that can be offered with regard to future efforts to perfect and improve the standard baking test. Possibilities for improvement in certain directions are indicated in this report. The outcome of these possibilities cannot be determined by any individual Research Fellow, but only by properly conducted collaborative tests involving different laboratories. The immediate task for future baking committees is to carefully select a few laboratories which have, beyond any doubt, adequate and uniform facilities for really meeting all specifications, in addition to a willingness to undertake such collaborative studies as may be designated by the committee even to the extent of purchasing an occasional item of equipment, such as, for instance, a set of "S-rolls." This necessitates, first of all, a survey of the situation in regard to facilities existing in cereal laboratories at the present time.

Recommendations and suggestions of a more specific and individual character are as follows:

Absorption. Absorption should be adjusted to suit each individual flour, with special care not to have doughs too slack or sticky. Absorption should always be reported on 15% moisture basis. Temperature of water should be such that doughs come from the mixing operation at 30° C. ± 2.0. This can be determined only by individual trials and experience.

Yeast. Modern bakers yeast seems to be reasonably uniform, and its degree of freshness is not as critical a factor as is generally assumed. It may be used satisfactorily either in water suspension, or otherwise.

Mixing Operation. The Hobart-Swanson mixer is decidedly superior to all other types encountered in these experiments. They are not yet on the market, but are expected to be available at an early date. The hand-mixing specification should remain as it now is until the Hobart-Swanson machine is generally available, in which event the mixing specification should be changed to 1 minute with the Hobart-Swanson mixer for the basic procedure. It is especially well adapted for studies relating to mixing tolerance, mechanical modification, etc.

Fermentation Bowls. The writers see no good reason for changing from the low form bowls as now specified. The specified dimensions of the bowl may be regarded as merely approximate, however. Slight deviations from these dimensions are of no serious consequence.

Punching Doughs. The use of "S-rolls," as described in this report, or of rolls similar in nature, is suggested as worthy of consideration and further study, both for punching and molding doughs. The S-rolls are practically identical only with the sheeting rolls of the Thomson Type G Roll Molder. They could doubtless be furnished by the Thomson Company, as a separate unit operated by hand, and at a cost well within the range of most laboratories. Until the advantages of the S-rolls can be either established or disproved by further collaborative tests, no change from the present prescribed method for punching doughs is recommended.

When using the short method, involving only 1½ hours of fermentation with the elimination of sugar from the formula, only one punch—and that at the end of an hour—is recommended. This operation is the same as specified for the first punch in the present basic procedure.

Molding and Panning. Here the same general problem is encountered as in punching the dough. The Thomson Roll Molder is excellent for the purpose, but its expense is a serious handicap to its general adoption. It should be possible to utilize some simpler and less expensive mechanical device. Such a device, designated throughout this report as the "S-rolls," is suggested as worthy of collaborative

study. It minimizes the personal element in pounding the gas out of the dough, which seems to be the main source of variability in hand molding. The use of a rolling pin running on tracks of specified thickness is also suggested as a possible improvement from the standpoint of variability among different technicians.

No immediate change in the prescribed molding procedure is recommended at this time. It is believed, however, that a decidedly improved procedure can be worked out along lines indicated, and suitable collaborative studies involving those principles are to be encouraged.

The use of a molding surface consisting of cotton or canvas belting, as first suggested by G. Moen, was studied and found highly satisfactory.

Baking. The writers favor the present oven specifications, as far as they go. Further specifications should eventually include: Rotating platform, oven dimensions, type and thickness of insulating material, nature and position of heating elements, and location of all parts with respect to each other. The elimination of top oven heat is favored by the writers as affording a comparatively better opportunity for differentiation among the top crust characteristics of various flours. Oven Y comes close to satisfying all necessary requirements.

For uniformity in baking results the use of an open pan of water in the oven is a worthwhile precaution.

Baking Pans. The spotless metal pans, now specified, leave much to be desired. They were found to be very difficult to break in, and unless thoroughly broken in and kept in constant use, their behavior was likely to be erratic. They required light greasing, claims to the contrary notwithstanding. It is hereby recommended that heavy tin be prescribed in place of the spotless metal.

Some study was devoted to low form tins as habitually used by Canadian technologists. These pans were found to have several distinct advantages, and although it is not now recommended that they replace the present style of pans in the standard procedure they are worthy of serious consideration and study.

Loaf Volume Measurement. Calibration of all loaf volume apparatus by some device such as the one suggested by Bailey (1930) is very strongly urged.

Supplementary Procedures. For reasons discussed in a preceding portion of this report, it is recommended that one of two things be done in future or revised written specifications for the standard baking test. Either several more "additional and supplementary" tests should be added to the four that are now mentioned or *none* should be specifically listed. In the latter case it is to be distinctly under-

stood that *any* supplementary test is as permissible as any other, and that no principle of scientific laboratory testing procedure is violated so long as not more than one variable at a time is introduced. It should be constantly realized, however, that no matter how strictly the test may conform to approved scientific principles, it can never be strictly *standard* until there is a common starting point, which in this case is the basic procedure.

The Short Method. Whether the so-called "short method," as hereinbefore described and discussed, should eventually become the basic procedure or merely another "supplementary" procedure, is a matter worthy of further consideration and further research.

The Bromate Differential Test. The magnitude of the familiar effect of $KBrO_3$, as used in the A. A. C. C. baking test, is somewhat reduced both by machine molding and punching, and especially by the use of the short method in which sugar is eliminated and the fermentation time reduced to $1\frac{1}{2}$ hours. In the latter case it is advisable to use KIO_3 instead of $KBrO_3$.

Acknowledgments

Acknowledgments are due, and are gratefully extended to those who have either loaned or contributed equipment or service during the active period of the A. A. C. C. Baking Research Fellowship. Among this group are the Thomson Machine Company, The Freas Thermo-Electric Company, the Dispatch Oven Company, Standard Brands, Inc., The Gooch Milling and Elevator Company, the Commander Larabee Company, the Rodney Milling Company, and numerous other milling organizations and individuals.

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APPLICATION OF THE VARIANCE ANALYSIS TO EXPERIMENTS IN CEREAL CHEMISTRY

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INTRODUCTION

The analysis of variance as developed by R. A. Fisher and his staff, of Rothamsted Experimental Station, has been applied to great advantage in the statistical reduction of experimental data. In Europe it is coming into very general use for the analysis of field plot experiments, and in other types of experimental work throughout the world its advantages are rapidly being recognized. In cereal chemistry experiments the analysis of variance seems to be particularly applicable, as there is a definite experimental variability which cannot be overcome by methods now in use; and the sorting out of this variability and allocating it to different sources is often the most serious problem with which the cereal chemist has to deal in generalizing from his results.

The best method of approach to an understanding of the analysis of variance is by means of examples, dealing at first with experiments of a very simple type and later with more complex ones. It will be observed, as such examples are studied, that the analysis of variance is a process of resolving the original mass of data into a comprehensive form, and one in which direct tests may be applied to the significance of any feature indicated by the data.

Division of Sums of Squares

In an experiment on the relative effects of hand and machine moulding of the dough in baking tests on wheat flour, results are given by Geddes et al. (1931). Table I contains a portion of the data obtained and this will do quite well to illustrate some of the fundamentals of the procedure.

TABLE I

FIVE PAIRS OF LOAF VOLUME DETERMINATIONS

Method of procedure	Loaf volume determinations in cc.				
Hand moulding	635	632	630	628	620
Machine moulding	572	570	570	580	572

One of the first and most important facts relative to an analysis of such data by the variance method is that the sum of the squares of the deviations of the individual determinations from the general mean may be split up into component parts. These sums of squares of the deviations are referred to, in brief, as the "sums of squares." The three logical component sums of squares of this experiment are, (1) due to variations in the volume of hand moulded loaves, (2) due to variations in the volume of machine moulded loaves, and (3) due to the difference between the mean of hand moulded and machine moulded loaves. We shall carry out the calculations of the total and the component sums of squares by the direct method of finding the actual deviations, squaring and summating. Most of this work is given in Table II.

TABLE II

DIRECT CALCULATION OF SUMS OF SQUARES

Method of procedure	Loaf volume determinations	Deviations from general mean (600.9)	Deviations squared	Deviations from mean of hand moulded loaves (629.0)	Squares of deviations, previous column
Hand moulding	635	34.1	1162.81	6.0	36.0
	632	31.1	967.21	3.0	9.0
	630	29.1	846.81	1.0	1.0
	628	27.1	734.41	1.0	1.0
	620	19.1	364.81	9.0	81.0
Totals	3145				128.0

Method of procedure	Loaf volume determinations	Deviations from general mean (600.9)	Deviations squared	Deviations from mean of machine moulded loaves (572.8)	Squares of deviations, previous column
Machine moulding	572	28.9	835.21	0.8	0.64
	570	30.9	954.81	2.8	7.84
	570	30.9	954.81	2.8	7.84
	580	20.9	436.81	7.2	51.84
	572	28.9	835.21	0.8	0.64
Totals	2864				68.80
Total sum of squares			8092.90		

These calculations give us: Total sum of squares = 8092.90 and

(1) Hand moulded loaves = 128.00,

(2) Machine moulded loaves = 68.80.

We still require the third sum of squares for the difference between hand and machine moulding so we find the means for the two groups and the deviations of these from the general mean.

Experimental procedure	Mean	Deviations from general mean	Squares of deviations, previous column
Hand moulding	629.0	28.1	789.61
Machine moulding	572.8	28.1	789.61
Total			1579.22

Now each squared deviation of 789.61 in the calculations just cited represents the squared deviation from the general mean of 5 individual determinations as a group. Consequently the sum of squares obtained must be multiplied by 5 and we obtain $(1579.22 \times 5) = 7896.10$. We now have the three sums of squares required.

(1) Hand moulded loaves	= 128.00
(2) Machine moulded loaves	= 68.80
(3) Differences between hand and machine moulding	= 7896.10
Total	= 8092.90

The total agrees *exactly* with that which was calculated directly from the deviations of each individual determination from the general mean.

Variance and Degrees of Freedom

It is obvious that sums of squares form the basis for the measurement of variability. Thus the standard deviation in a very large population is defined as $\sqrt{\frac{S(x - \bar{x})^2}{n}}$, where $S(x - \bar{x})^2$ is the sum of squares and n is the number of determinations. In a definitely limited population the standard deviation is $\sqrt{\frac{S(x - \bar{x})^2}{DF}}$, where DF represents the number of degrees of freedom and can usually be equated to $n - 1$ where n is the number of determinations. The sums of squares obtained in our calculations can, therefore, be used to determine standard deviations by dividing by the number of degrees of freedom, and these should show wherein the greater part of the variability of the experiment lies. In the variance method of analysis, however, it is not necessary to proceed as far as the extraction of the square roots. We merely divide the sums of squares by the corresponding number of degrees of freedom and obtain squared standard deviations only. Any such squared standard deviation is referred to as a *variance* and is in reality a mean of the squared deviations. The point of this is that variances are just as readily compared as standard deviations and the additional calculations are unnecessary.

We proceed, therefore, to determine the number of degrees of

freedom corresponding to each sum of squares and to the calculation of variances. There is a total of 10 determinations so that there will be a total of $(10 - 1) = 9$ degrees of freedom for the entire experiment. There are 5 hand moulding and 5 machine moulding determinations so that each of these will contribute 4 degrees of freedom. There are 2 determinations for the comparison of hand and machine moulding so that this comparison will contribute 1 degree of freedom. The total for the component parts $(4 + 4 + 1) = 9$, agrees with that obtained by $(10 - 1) = 9$. We see, therefore, that there is a definite relationship not only between the sums of squares for the entire experiment but also between the degrees of freedom. Using the numbers we have calculated instead of algebraic symbols we have the following two equations for our experiment:

	Total		Hand moulding		Machine moulding		Comparison, hand and machine moulding
Sum of squares	8092.90	=	128.00	+	68.80	+	7896.10
Degrees of freedom	9	=	4	+	4	+	1

In complex experiments the degrees of freedom are often slightly difficult to sort out and it is of considerable assistance to know that the sum of the degrees of freedom as sorted out within the experiment can always be equated to the total.

The calculation of the variances is best carried out in tabular form as in Table III.

TABLE III

TABULAR CALCULATION OF VARIANCES

Source of variance	Sum of squares	Degrees of freedom	Variance	
Hand moulded loaves	128.00	4	32.00	(V_1)
Machine moulded loaves	68.80	4	17.20	(V_2)
Comparison, hand and machine moulding	7896.60	1	7896.10	(V_3)
Total	8092.90	9	899.21	(V_4)

The most fundamental concept in respect to the analysis of variance is that each variance calculated is an estimate of the total variance for the experiment. In an experiment such as the one we are discussing if the 10 loaf volumes were taken entirely at random from a large number of such determinations we would expect on repeating the procedure a large number of times that the three estimates of variance would all vary around the variance for the entire experiment. This is actually the case as has been proved theoretically and experimentally. Fisher (1924) has worked out the distributions which enable us to determine

in what percentage of the cases a given deviation of a variance from the true value will occur. If the digression of an estimate of variance is well beyond that to be expected on the basis of chance we say that it is significant. We conclude that it represents in the data some systematic effect; something that is not due to chance variation.

The Z Test

In the experiment on hand and machine moulding one of the things on which we wish to obtain information is the effect of machine moulding in reducing variability in loaf volume. This resolves itself into making a comparison of the two estimates of variance, 32.00 for hand moulding, and 17.20 for machine moulding. It brings us to the mechanics of making such comparisons, generally known as the application of Fisher's Z test. We have to find the value of Z which is one half the difference between the natural logarithms of the two estimates of variance. The calculations in detail as they are commonly carried out are as follows:

	Variance	Degrees freedom	Common log. of variance	½ natural log. = common log. × 1.1513	Difference = Z
Hand moulding	32.00	$n_1 = 4$	1.5051	1.7328	0.3104
Machine moulding	17.20	$n_2 = 4$	1.2355	1.4224	

We now examine Fisher's tables (1931) of the 5% points of the distribution of Z and locate the value corresponding to $n_1 = 4$, and $n_2 = 4$, which is 0.9272. In this experiment, therefore, a value of Z equal to or greater than 0.9272 would be obtained by chance in 5% of the cases and consequently we cannot consider that a Z value of 0.3104 even approaches significance. This, of course, is a very logical result as with such a small experiment differences between estimates of variance have to be very large before they can be considered significant. As more degrees of freedom are available for a comparison the differences necessary to indicate significance become less. If we examine the table of 5% points for $n_1 = 24$ and $n_2 = 24$ we note that the 5% point is 0.3425. Thus, if our results had been based on 25 loaves for each method, the result could have been considered as approaching significance.

Another point in the experiment we wish to test is the significance of the difference between the means of hand moulded and machine moulded loaves. It was this difference that contributed to the third estimate of variance (7896.10) and consequently we can test the significance of the mean difference by testing the significance of this

corresponding estimate of variance. The question has to be settled, however, as to what variance V_3 must be compared with. Obviously it should not be compared with the total variance V_4 as it has itself contributed to the magnitude of this variance. As to V_1 and V_2 these both represent random error, i.e., they are both due to errors that are beyond control, but if we have to take either one of them to compare with V_4 it is obviously impossible to decide which one should be selected. The logical conclusion is to pool V_1 and V_2 to form a new estimate of the random error throughout the test and this should form a logical basis for comparison with the estimate of variance due to differences between hand and machine moulding. To do this we add the sum of squares and the degrees of freedom for V_1 and V_2 obtaining, sum of squares = 196.80, and degrees of freedom = 8. A new variance table is now formed as in Table IV below.

TABLE IV

ANALYSIS OF VARIANCE

Source of variance	Sum of squares	Degrees of freedom	Variance
Comparison, hand and machine moulding	7896.10	1	7896.10
Random error	196.80	8	24.60
Total	8092.90	9	

Then comparing the estimates of variance we have:

	Variance	Degrees freedom	Common log.	$\frac{1}{2}$ natural log.	Z	5% point
Comparison, hand and machine moulding	7896.10	$n_1 = 1$	3.8974	4.4871	2.8858	0.8355
Random error	24.60	$n_2 = 8$	1.3909	1.6013		

In entering the table of 5% points it must always be remembered that n_1 corresponds to the larger variance. Thus $n_1 = 1$ corresponds here to the variance 7896.10. If we had entered the table in the wrong direction using $n_1 = 8$, $n_2 = 1$, we should have obtained an erroneous 5% point of 2.7380.

The Z value of 2.8857 is far beyond the 5% point so we can conclude that the mean difference due to hand and machine moulding is quite significant. A value of Z which is just equal to the 5% point corresponds to odds of 19 to 1 and for our result we know nothing so far except that the odds are much greater than 19 to 1. To obtain further information on the odds we can use Fisher's table of the 1% points of the distribution of Z. This table gives us a 1% point of 1.2106 for

$n_1 = 1$, and $n_2 = 8$. The odds are, therefore, greater than 99 to 1 that the difference in loaf volume between machine and hand moulding is significant.

Control of Error

The above would complete our analysis of the experiment as the data have been set up, but for the sake of simplicity we have not represented the experiment exactly as it was carried out. In the actual baking one hand moulded loaf and one machine moulded loaf was baked from the same mixing of flour and placed together in the oven, consequently the data can be arranged in pairs of loaf volumes and any variations due to influences affecting the individuals of each pair in a like manner can be theoretically removed from the estimate of random error. As far as the comparison of the mean loaf volumes for hand and machine moulding is concerned this is logical, as such error is irrelevant to the comparison; it affects the volumes for both hand and machine moulding in exactly the same way and consequently does not enter into the variability of the difference between them. This means that another sum of squares and another estimate of variance must be determined, which is essentially that due to deviations of the means of the paired values from the general mean. We now set up the data in pairs as in Table V, and calculate the means

TABLE V

Calculation of Sum of Squares for Paired Values

Hand moulding	Machine moulding	Mean	Deviations from general mean	Squares of previous column
635	572	603.5	2.6	6.67
632	570	601.0	0.1	0.01
630	570	600.0	0.9	0.81
628	580	604.0	3.1	9.61
620	572	596.0	4.9	24.01
				Total = 41.20

of the pairs, deviations from the general mean, and squares of the deviations. Here each mean represents 2 determinations, therefore, we have to multiply the calculated sum of squares by 2. This gives $(41.20 \times 2) = 82.40$ as the total sum of squares. There are 5 mean determinations entering into this sum of squares so that the corresponding number of degrees of freedom must be 4. This sum of squares we have calculated must come out of the total for error of 196.80 (Table IV), and similarly the 4 degrees of freedom must come out of the 8 degrees of freedom formerly apportioned to random error. This changes the analysis in Table IV to the analysis given below in Table VI.

TABLE VI

COMPLETE ANALYSIS OF VARIANCE

Source of variance	Sum of squares	Degrees of freedom	Variance
Comparison, hand and machine moulding	7896.10	1	7896.10
Variability of pairs	82.40	4	20.60
Random error	114.40	4	28.60
Total =	8092.90	9	

This is not a fortunate example to have selected to demonstrate the value of controlling the error by pairing arrangements as we have actually increased the variance for error from 24.60 to 28.60, but from another standpoint it is a very fortunate example as it teaches one of the most important principles encountered in dealing with the statistics of small samples. The exercise of control over experimental error as we attempted to do here always results in a loss of degrees of freedom, and if the reduction of the sum of squares for error is not more than proportionate to the corresponding loss of degrees of freedom there is no gain in precision, and as in this case there may be an actual loss in precision. For the loss in precision is not only that represented by the higher estimate of error; there is a loss of 4 degrees of freedom and consequently the 5% point will be higher and a greater difference between the estimates of variance being compared is necessary in order to indicate significance. This becomes perfectly clear as the Z test is applied. Repeating the test on page 244 using the new estimate of error we have:

	Variance	Degrees freedom	Common log.	$\frac{1}{2}$ natural log.	Z	5% point
Comparison, hand and machine moulding	7896.10	1	3.8974	4.4871	2.8103	1.0212
Random error	28.60	4	1.4564	1.6768		

The Z value is not only lower than in the previous test but the 5% point is much higher and while the result is still quite significant there is an obvious loss in precision. In such cases as this the analysis giving the greatest precision should be used and the other one discarded. This is a perfectly logical procedure as in the second case we have imposed on the data in the mode of our analysis a fictitious condition based on the assumption that the pairs of values would vary together to an appreciable extent. The analysis shows that there was no such effect and we should obviously go back to the first method of analysis.

Methods of Analysis

This completes the analysis of our simple experiment but we have used methods of calculating the sums of squares which are more cumbersome than is necessary. The methods used show the exact meaning of the sums of squares and the estimates of variance calculated from them, but much more rapid methods are available especially for machine calculation. In order to demonstrate these we shall set up the data again in the form of a table but in order to reduce the amount of calculation we subtract a uniform value from each of the loaf volume figures. This process is known as "coding." In this instance 550 is a convenient number to subtract from each value. Thus we have Table VII in which the grand total and the sub-totals in both directions have been computed and an algebraic symbol is given to each total.

TABLE VII

RAW DATA IN TABULAR FORM

	Coded loaf volumes in cc.					Totals
Hand moulding	85	82	80	78	70	$395 = t_H$
Machine moulding	22	20	20	30	22	$114 = t_M$
Totals	107	102	100	108	92	$509 = t_G$
	t_1	t_2	t_3	t_4	t_5	

Assuming that an analysis as on page 242 is to be carried out, 4 sums of squares will be required:

(1) Total.
(2) Due to variability within hand moulded loaves.
(3) Due to variability within machine moulded loaves.
(4) Comparison, machine and hand moulding.

If x is taken to represent any single determination these sums of squares are given by the following formulae:

$$(1) = S(x)^2 - \frac{t_g^2}{n_g'} = (85^2 + 82^2 + \cdots + 22^2) - \frac{(509)^2}{10}$$
$$= 34001 - 25908.1 = 8092.9,$$

$$(2) = S(x_h)^2 - \frac{t_h^2}{n_h'} = (85^2 + 82^2 + \cdots + 70^2) - \frac{(395)^2}{5}$$
$$= 31333 - 31205 = 128.0,$$

where x_h represents hand moulded loaves only;

$$(3) = S(x_m)^2 - \frac{t_m^2}{n_m'} = (22^2 + 20^2 + \cdots + 22^2) - \frac{(114)^2}{5}$$
$$= 2668 - 2599.2 = 68.8,$$

where x_m represents machine moulded loaves only;

$$(4) = \frac{(t_h - t_m)^2}{n_g'} = \frac{(395 - 114)^2}{10} = 7896.1.$$

Adding (2), (3), and (4) gives 8092.9 which is a complete check on the calculations.

It is not necessary to remember these formulae but only the

principles on which they are built up. The right formula for the calculation of a given sum of squares can then be written down immediately and in actual practice it is not even necessary to write down the formula as the calculations can be proceeded with directly. To find the sum of squares for a set of values like 85, 82, 80, 78, and 70, we merely have to square each value and summate, then subtract the square of the total of these values divided by the number. Thus the sum of squares is as follows:

$$(85^2 + 82^2 + 80^2 + 78^2 + 70^2) - \frac{(395)^2}{5}.$$

To find the sum of squares represented by a series of sub-totals such as 107, 102, 100, 108, and 92, we go through exactly the same procedure except that the squares of the sub-totals are divided by the number of values entering into each sub-total. Here each sub-total such as 107 is the sum of 2 values, 85 and 22, and consequently the sum of squares will be:

$$\frac{(107^2}{2} + \frac{102^2}{2} + \frac{100^2}{2} + \frac{108^2}{2} + \frac{92^2)}{2} - \frac{(509)^2}{10}.$$

The square of each individual total as well as the grand total is divided by the number of values entering into it where each value results from a single determination. Of course, in the above case the division by 2 is carried out at one step so that we have:

$$\frac{(107^2 + 102^2 + \cdots\cdots\cdots\cdots\cdots + 92^2)}{2} - \frac{(509)^2}{10}.$$

But in some cases the sub-totals are not formed from the addition of the same number of values so that each division must be carried out separately. Suppose we have the following figures:

				Grand total
Total	122	683	205	1010
Number of determinations entering into the total	4	9	5	18

The sum of squares represented by these figures will be:

$$\frac{(122^2}{4} + \frac{683^2}{9} + \frac{205^2)}{5} - \frac{(1010)^2}{18}.$$

It will also be observed from the formula on page 247 that for the comparison of hand and machine moulding the sum of squares was determined from the two totals 395 and 114 and that in this case a very simple formula was used. On the principles given above the sum of squares for these two values would be:

$$\frac{(395^2 + 114^2)}{5} - \frac{(509)^2}{10} \quad \text{or} \quad \frac{t_h^2 + t_m^2}{5} - \frac{(t_h + t_m)^2}{10};$$

but it can be verified easily that this is equal to:

$$\frac{(t_h - t_m)^2}{10} = \frac{(395 - 114)^2}{10},$$

which is a worth while short cut in computation and can always be employed if the two sub-totals represent the same number of additions.

Proceding to the analysis of variance in which the variability of the loaf volumes in pairs was removed from the random error it is obvious that the sum of squares required will be given by the totals t_1, t_2, t_3, t_4, and t_5. This is the sum of squares we have already discussed.

$$\frac{(107^2 + 102^2 + 100^2 + 108^2 + 92^2)}{2} - \frac{(509)^2}{10} = 25990.5 - 25908.1 = 82.4.$$

And this sum of squares when used in the analysis of variance must be deducted from the 196.80 formerly obtained as the sum of squares representing random error.

Degrees of Freedom

In a study of the analysis of variance some consideration should be given to the use of degrees of freedom. To those not acquainted with the underlying mathematical principles this may seem to be a somewhat mystifying procedure but actually it is very simple and the necessity for its use may be demonstrated easily. As Fisher (1931) has pointed out, if we have four cells in a table to fill up with numbers and the total of these four numbers is fixed, there are only three cells that we can fill up arbitrarily. When these are filled the number to be placed in the remaining cells is fixed because the total is fixed. This led Fisher to the use of the term "degrees of freedom" and in the above case with four cells to be filled there could only be three degrees of freedom.

Similarly in an actual analysis of variance it is possible to demonstrate the necessity for the use of degrees of freedom, and for this purpose we shall take 16 loaf volume determinations drawn at random from a group of 50 determinations all made on the same day, with the same flour, and by the same baker. These we shall divide up into four groups of four each, representing the groups by the letters *A*, *B*, *C*, and *D*. The coded data are given in Table VIII.

TABLE VIII

CODED DATA FOR SIXTEEN LOAF VOLUME DETERMINATIONS TAKEN AT RANDOM AND ARBITRARILY DIVIDED INTO FOUR GROUPS

	A	*B*	*C*	*D*	
	30	20	32	40	
	35	20	52	52	
	52	40	52	25	
	20	40	40	20	
Totals	137	120	176	137	Grand total = 570

The total sum of squares is:

$$(30^2 + 20^2 + \cdots\cdots\cdots + 20^2) - \frac{(570)^2}{16} = 22590 - 20306.25 = 2283.75$$

and represents 15 degrees of freedom.

The 5 logical component sums of squares are (1) within A, (2) within B, (3) within C, (4) within D, and (5) between the means of A, B, C, and D.

The calculations are:

(1) $$(30^2 + 35^2 + \cdots 20^2) - \frac{137^2}{4} = 5229.00 - 4692.25 = 536.75,$$

(2) $$(20^2 + 20^2 + \cdots 40^2) - \frac{120^2}{4} = 4000 - 3600 = 400.00,$$

(3) $$(32^2 + 52^2 + \cdots 40^2) - \frac{176^2}{4} = 8032 - 7744 = 288.00,$$

(4) $$(40^2 + 52^2 + \cdots 20^2) - \frac{137^2}{4} = 5329 - 4692.25 = 636.75,$$

(5) $$\frac{137^2 + 120^2 + \cdots + 137^2}{4} - \frac{(570)^2}{16} = 20728.5 - 20306.25 = 422.25.$$

The sums of squares (1), (2), (3), (4), and (5), when totalled give 2283.75 which checks perfectly with the direct calculation of the total sum of squares. Each of the components represent 3 degrees of freedom so that we have a total of 15 as worked out directly. The variance calculations are done in the usual tabulated form, in Table IX.

TABLE IX

CALCULATION OF THE ESTIMATES OF VARIANCE

	Sourc of variance	Sums of squares	Degrees freedom	Variance
(1)	Within A	536.75	3	178.92
(2)	" B	400.00	3	133.33
(3)	" C	288.00	3	96.00
(4)	" D	636.75	3	212.25
(5)	" A, B, C, and D.	422.25	3	140.75
	Total	2283.75	15	152.25

Referring again to the fundamental principle of variance analysis, each of the 5 variances calculated as above for a portion of the data are *estimates* of the total variance of the experiment 152.25. If as in this case the five estimates of variance are based on the same number of degrees of freedom the average of the estimates will be exactly equal to the total variance. This follows of course, because the sum of all the degrees of freedom used in calculating the 5 estimates of variance is equal to the number of degrees of freedom used in calculating the total variance. If we throw A, B, C, and D, together we have the

analysis in Table X in which 152.25 is the average of 155.12 and 140.75 weighted according to the degrees of freedom.

TABLE X

ANALYSIS OF VARIANCE, A, B, C, AND D COMBINED

Source of variance	Sum of squares	Degrees freedom	Variance
(1) Within A, B, C, and D	1861.50	12	155.12
(2) Between A, B, C, and D.	422.25	3	140.75
Total	2283.75	15	152.25

These tables may be used to show the inaccuracies induced if degrees of freedom are not used. In Table XI for example, if degrees of freedom are not used each of the component sums of squares will be divided by 4 instead of 3 and the total sums of squares will be divided by 16. Also, the estimates of variance are shown calculated in two ways and these are given in per cent of the calculated total variance.

TABLE XI

ESTIMATES OF VARIANCE CALCULATED WITH AND WITHOUT USING DEGREES OF FREEDOM

Sums of squares	Degrees freedom	Variance	Estimated variance in per cent of total	Number of determinations	Variance incorrectly determined	Previous column in per cent 142.73
536.75	3	178.92	117.52	4	134.19	94.02
400.00	3	133.33	87.57	4	100.00	70.06
288.00	3	96.00	63.05	4	72.00	50.44
636.75	3	212.25	139.41	4	159.19	111.53
422.25	3	140.75	92.45	4	105.56	73.96
2283.75	15	152.25	Av. = 100.00	16	142.73	Av. = 80.00

In the second case where degrees of freedom are not used the average of the 5 estimates of variance is only 80 per cent of the total variance. This brings out the absolute necessity of the use of degrees of freedom. Otherwise the variance will *always* be *under estimated.* But the danger is not at its greatest in analyses of this kind where estimates of variance that are based on the same number of degrees of freedom are being compared. The real danger comes in comparing estimates of variance based on different numbers of degrees of freedom, as the variance based on the smaller number of degrees of freedom will obviously be depressed to a greater extent than the other. In Table XI, for example, the average of the 5 estimates of variance when degrees of freedom are not used is 80%, i.e., they have all been underestimated to the extent of 20%. Now the total variance when

degrees of freedom are not used is 142.73 which is 93.75% of 152.25, i.e., it has been underestimated to the extent of 6.25%. In comparing two estimates of variance, therefore, one based on 16 determinations (15 *DF*) and the other based on 4 determinations (3 *DF*), the latter will be underestimated 20% and the former only 6.25%. Consequently, such a comparison is entirely unfair. It is based on an entirely erroneous mathematical foundation.

Another source of error in the incorrect use of degrees of freedom other than that mentioned above is obvious from an inspection of Fisher's tables of the 5% and 1% points of the distribution of Z. In comparing the two estimates of variance in Table IV, for example, we would find the 5% point for $n_1 = 1$, $n_2 = 8$, which is 0.8355. If we had not considered the degrees of freedom but had used the tables as though they were based on the number of mean determinations we would have looked up the 5% point for $n_1 = 2$, and $n_2 = 9$, which is 0.7242 and this value would actually be exceeded in the experiment in question in more than 5% of the cases, due to random variation. Consequently this also would be a source of error.

Application of the Analysis of Variance

The analysis of variance can be applied to a great variety of research problems and in each case the method of application presents certain new features. However, these new features present no serious difficulties if the principles and methods of calculation are clearly understood. Practice in application to different kinds of problems is obviously the best means of becoming familiar with the analysis of variance in general, and with each application it becomes easier to work out analyses for new problems. Two examples are given here, that are more or less typical and will serve to emphasize the principles previously discussed.

Wheat Variety Tests

When a series of varieties are to be tested for the baking quality of the flour they produce, the usual practice is to bake two loaves of each and average the results of the two loaf volume determinations. It is conceivable that the variations in loaf volume encountered in the baking test may be so great that the averages obtained may not mean very much. What we mean by this in statistical terms is that the variability induced by chance variations in the baking technique or other variable influences may be just as great as the variability resulting from real differences between the flours. It is highly desirable, therefore, that some means of determining the extent of the random error be used and that a comparison be made between this random

error and the variations due to real differences. The analysis of variance furnishes the mechanism for such a test and furthermore a standard error or probable error may be obtained which can be used for comparing the flours in detail.

The data given in Table XII were obtained by the Associate Committee on Grain Research, of the National Research Council of Canada, in a study of wheat varieties and have been set up in the form in which the primary calculations are carried out. The actual loaf volumes of the duplicate determinations are given in the first two columns, and in the third and fourth columns these values have been coded. The duplicate determinations are referred to as x and y for convenience in describing the variance calculations.

When facing a set of results of the kind given in Table XII the first order of procedure is to divide up the total degrees of freedom into logical components. Each of these will give a corresponding estimate of variance, and in the final picture the relative order of these estimates of variance will indicate the nature of the results that have been obtained. In this experiment 46 loaves were measured so that we have a total of 45 degrees of freedom for the experiment. There are 23 varieties giving 23 mean determinations, each mean being based on two loaf volumes. Thus, differences between varieties represent 22 degrees of freedom. Now error is obviously reflected by the differences between duplicates. If these values were exactly alike there would be no error whatsoever. Each pair of determinations contributes 1 degree of freedom, giving 23 altogether. The division of the total degrees of freedom results then in:

(1) Differences between varieties	$= 22\ DF$
(2) Differences between duplicate loaf volumes (error)	$= 23\ DF$
Total	$= 45\ DF$

The sums of squares must now be worked out according to this scheme. Actually only two of the sums of squares need to be calculated. If we find (1) and the total, (2) can be obtained by subtracting (1) from the total. As a general practice, however, for analyses of this kind it is desirable to calculate the three sums of squares directly, as in this way we get a complete check on the calculations. In many cases the sum of squares for error is very difficult to compute directly and is then obtained by subtracting the other sums of squares from the total.

To calculate the total sum of squares:

$$(190^2 + 186^2 + 238^2 + \cdots + 102^2) - \frac{(9054)^2}{46} = 1886306.0 - 1782063.3$$
$$= \underline{104242.7}$$

For varieties:

$$\frac{376^2 + 482^2 + \cdots + 226^2}{2} - \frac{(9054)^2}{46} = 1883967.0 - 1782603.3$$
$$= \underline{101903.7}$$

The sum of squares for error will be

$$104242.7 - 101903.7 = \underline{2339.0}$$

if obtained by differences. To calculate it directly we can use $\frac{1}{2}S(x-y)^2$ which is a general formula for the sum of squares representing differences between paired values. The values of $(x-y)$ have been prepared for this purpose in Table XII and we merely square these, summate and divide by 2. $\frac{1}{2}S(x-y)^2 = \frac{4678}{2} = 2339.0$; and we have a perfect check on previous calculations.

TABLE XII

LOAF VOLUMES OBTAINED IN A VARIETY TEST AND PREPARATION OF THE DATA FOR A VARIANCE ANALYSIS.

	Duplicate loaf volumes cc.		Coded loaf volumes cc.		Data for calculations		Mean loaf volume in cc.	Means in per cent of general mean
Variety	x	y	x	y	$x-y$	$x+y$		
1	590	586	190	186	4	376	588.0	81.8
2	638	644	238	244	−6	482	641.0	107.4
3	578	585	178	185	−7	363	581.5	97.4
4	548	566	148	166	−18	314	557.0	93.3
5	620	648	220	248	−28	468	634.0	106.2
6	588	609	188	209	−21	397	598.5	100.3
7	640	644	240	244	−4	484	642.0	107.6
8	610	610	210	210	0	420	610.0	102.2
9	572	576	172	176	−4	348	574.0	96.2
10	636	650	236	250	−14	486	643.0	107.7
11	658	650	258	250	8	508	654.0	109.6
12	586	598	186	198	−12	384	592.0	99.2
13	582	590	182	190	−8	372	586.0	98.2
14	526	530	126	130	−4	256	528.0	88.5
15	638	644	238	244	−6	482	641.0	107.4
16	492	472	92	72	20	164	482.0	80.8
17	598	600	198	200	−2	398	601.0	100.7
18	584	604	184	204	−20	388	594.0	99.5
19	580	584	180	184	−4	364	582.0	97.5
20	666	650	266	250	16	516	658.0	110.2
21	558	562	158	162	−4	320	560.0	93.8
22	654	684	254	284	−30	538	669.0	112.1
23	524	502	124	102	22	226	513.0	86.0
			4466	4588	−122	9054		

General mean = 596.8.
Standard error of one variety = 7.13.
Minimum significant difference = 21.4.
Minimum significant difference in per cent = 3.6.

The variance calculations and the Z test can now be combined in a single table as in Table XIII. In this table the writing down of the common logarithms has been omitted. The obvious test in this

TABLE XIII

ANALYSIS OF VARIANCE AND Z TEST

Source of variance	Sum of squares	Degrees freedom	Variance	½ loge	Z	5% point
Differences between varieties	101903.7	22	4631.99	4.2204	1.9094	0.3521
Error	2339.0	23	101.70	2.3110		
Total	104242.7					

instance is the comparison of the variance for variety differences with that for error as this will establish the presence or absence of real differences between the varieties or it will indicate whether or not the test has been sufficiently accurate to demonstrate any differences that may exist. The Z value is, of course, the difference between the two numbers 4.2204 and 2.3110 each of which is one-half of the natural logarithms of the corresponding variances. A Z of 1.9094 is obviously highly significant in this case as the 5% point is only 0.3521. Clearly the test has shown that real differences between the varieties exist and, therefore, a more detailed examination of the results is justified.

In looking up the 5% point for $n_1 = 22$, and $n_2 = 23$, a word of explanation may be necessary. Fisher's tables give 5% points for $n_1 = 1, 2, 3, 4, 5, 6, 8, 12, 24, \infty$ and, therefore, beyond 6 it may be necessary to interpolate if the 5% point is to be obtained accurately. For n_2 Fisher's tables give all of the values from 1 to 30, and the next set given are for 60 and the last for ∞. It is often necessary to interpolate in this direction also. In order to facilitate interpolation Fisher has given the values of n_1 for 6, 8, 12, 24, and ∞ which are in harmonic progression and this facilitates interpolation, as for these values of n_1 the 5% points are approximately in arithmetic progression. Details with regard to methods of interpolation are given in Fisher's book and need not be taken up here with the exception that the method of calculating the 5% point for $n_1 = 22$ and $n_2 = 23$ may be taken as an example.

We first locate in the table the 5% points for $n_1 = 12$, $n_2 = 23$, and $n_1 = 24$, $n_2 = 23$. These are 0.3950 and 0.3478. We then calculate:

$$5\% \text{ point} = .3478 + (.3950 - .3478) \times \frac{(24 - 22)}{(22)} = .3521.$$

The method is obvious except for the last factor $(24 - 22)/(22)$. This factor depends on the position in the table for which we are inter-

polating. If we let n_x represent the degrees of freedom to which we are interpolating as for 22 in the above example, the following are all of the factors required:

Interpolation between	Factor
7 to 8	.4286
8 to 12	$\frac{2(12 - n_x)}{n_x}$
12 to 24	$\frac{24 - n_x}{n_x}$
24 to ∞	$\frac{24}{n_x}$
30 to 60	$\frac{60 - n_x}{n_x}$
60 to ∞	$\frac{60}{n_x}$

The analysis in Table XIII gives 101.70 as the variance due to random error, and this, as already pointed out, is the squared standard deviation. The standard deviation then is $\sqrt{101.70} = 10.08$ and the probable error is $10.08 \times .6745 = 6.80$. The standard error of the mean for any one variety is $10.08/\sqrt{2} = 7.13$ and the standard error of the difference between any two means is $7.13 \times \sqrt{2} = 10.08$. If we let $7.13 = SE$ it is convenient to take $3 \times 7.13 = 21.39$ as the minimum difference between any two means that can be considered significant. A difference, if significant, should be at least twice its standard error, and in this case at least $2 \times 10.08 = 20.16$. This figure can be obtained roughly by taking three times the standard error of the experiment. In general, if SD is the standard deviation of an experiment and SE is the standard error of the mean of one variety calculated by $SE = SD/n$, where n represents the number of determinations entering into the mean value, a minimum significant difference can be taken as $3 \times SE$. The actual minimum significant difference is $SD/n \times \sqrt{2} \times 2$ and this is taken approximately as $SD/n \times 3$.

For an easy inspection of differences and their significance, percentage values are very convenient. Thus, in Table XII the means for each variety are given in actual values and in per cent of the general mean. In the actual values a difference of about 21 cc. can be considered significant, and in the percentage values a difference of 3.6%.

Variety Tests in Different Districts

It is well known that soil and climate have a decided effect upon wheat quality, and useful information should be furnished by an experiment in which tests are made on the quality of a group of wheat varieties grown under different environmental conditions. The data given in Table XIV were obtained for this purpose by the Associate

TABLE XIV

LOAF VOLUMES IN DUPLICATE TESTS FOR 14 VARIETIES GROWN IN 10 DISTRICTS THROUGHOUT WESTERN CANADA

Data coded by subtracting 400 from the actual loaf volumes

Variety	Districts																				Variety Totals
	Swift Current		Indian Head		Rosthern		Winnipeg		Brandon		Scott		Saskatoon		Lethbridge		Edmonton		Lacombe		
1	75	45	155	140	165	145	190	186	182	178	228	244	250	250	232	228	295	312	218	214	3932
2	125	132	200	185	150	140	238	244	196	194	266	274	308	326	290	310	190	200	190	194	4352
3	70	10	100	80	155	140	178	185	200	202	224	218	278	282	290	292	228	240	220	246	3838
4	15	20	130	150	85	90	148	166	92	116	70	96	140	126	76	90	188	208	104	120	2230
5	280	290	195	160	105	120	220	248	162	170	290	272	230	234	234	255	234	212	178	192	4281
6	155	147	175	190	130	140	188	202	196	224	260	260	194	190	246	270	260	240	236	220	4123
7	140	145	360	355	175	180	240	244	236	240	280	278	194	176	362	385	216	238	242	222	4908
8	140	140	185	175	100	105	210	210	180	162	240	248	264	264	340	318	240	250	202	200	4173
9	135	105	180	195	155	165	172	176	180	192	196	188	240	232	310	305	275	300	268	262	4231
10	135	100	110	120	200	210	236	250	170	180	178	176	240	220	246	265	186	178	200	202	3802
11	135	130	185	195	185	210	186	198	220	224	298	288	265	278	272	300	240	250	214	232	4505
12	222	225	235	260	200	195	182	190	182	190	218	230	350	340	268	262	280	276	244	250	4799
13	120	90	115	130	95	105	126	130	172	176	224	244	208	206	340	314	150	154	142	150	3391
14	185	165	185	195	230	240	238	244	198	210	312	288	288	278	218	208	244	250	254	238	4668
District totals	3676		5040		4315		5625		5224		6588		6851		7526		6534		5854		57,233

Committee on Grain Research, of the National Research Council of Canada. Fourteen varieties were grown at 10 points throughout Western Canada and 20 loaves were baked from the flour of each variety, 2 from each district. These data enable us to study the variability due to districts, to varietal differences, and that due to the differential effect of districts on varieties. The latter effect is known in terms of the analysis of variance as an interaction.

Since there was a total of 280 loaves baked, the first step in the analysis is the allocation of the total of 279 degrees of freedom. We have $13(DF)$ for varieties, and $9(DF)$ for districts, leaving $257(DF)$ for error and the interaction of varieties and districts. The latter may be separated by two methods which must, of course, give the same results. The easiest method in general is to follow the rule that the number of degrees of freedom supplied by an interaction is the product of the degrees of freedom corresponding to the interacting factors. In this case we have $13(DF)$ for varieties, and $9(DF)$ for districts, and there must be $(9 \times 13) = 117(DF)$ for the interaction. This leaves $(257 - 117) = 140(DF)$ for error. We may, if we wish, determine the degrees of freedom for error independently as there are 140 pairs of duplicate loaf volume determinations each of which supplies one degree of freedom. Thus, there is a total of $140(DF)$ for error, and this leaves $(257 - 140) = 117(DF)$ for the interactions. The outline of the analysis based on the degrees of freedom will be therefore:

Varieties	13 *DF*
Districts	9 *DF*
Interaction (districts × varieties)	117 *DF*
Error	140 *DF*
Total	279 *DF*

For the calculation of the sums of squares we require the 10 district totals, the 14 variety totals, the 140 totals of duplicate loaf volume determinations, and the grand total. After decoding the values in Table XIV by subtracting 400 from each one we determine:

(1) Total sum of squares:

$$\left[S(x)^2 - \frac{T^2}{280} \right] = 12894357 - \frac{(57233)^2}{280} = 1195727.4,$$

where x represents the individual loaf volume determination and T is the grand total.

(2) Sums of squares for differences between varieties:

$$S(T_v^2) - T^2 \; 239903251 - (57233)^2 = 296532.9,$$

$$\frac{S(T_v^2)}{20} - \frac{T^2}{280} = \frac{239903251}{20} - \frac{(57233)^2}{280} = 296532.9,$$

where T_v is the total for one variety.

(3) Sum of squares for differences between districts:

$$\frac{S(T_d)^2}{28} - \frac{T}{280} = \frac{340405695}{28} - \frac{(57233)^2}{280} = 458716.6,$$

where T_d is the total for one district.

(4) Sum of squares for interaction:

$$\frac{S(x+y)^2}{2} - \frac{T^2}{280} - (2) - (3),$$

where $(x + y)$ is the sum of one pair of duplicate determinations and (2) and (3) are the sums of squares for varieties and districts already calculated. The actual figures are: 1178912.9 − 296532.9 − 458716.6 = 423663.4.

(5) Sum of squares for error is the total sum of squares less (2), (3), and (4):

$$1195727.4 - (296532.9 + 458716.6 + 423663.4) = 16814.5.$$

The error sum of squares may be checked by calculating $\frac{1}{4}S(x - y)^2$.

Setting up the analysis of variance we have Table XV. The Z values given are those for comparing the corresponding estimate of

TABLE XV
ANALYSIS OF VARIANCE

Source of variance	Sum of squares	Degrees freedom	Variance	$\frac{1}{2}$ log$_e$	Z	5% point
Differences between districts	458716.6	9	50968.51	5.4195	3.0253	0.3317
Differences between varieties	296532.9	13	22810.22	5.0175	2.6233	0.2894
Interaction (districts varieties)	423663.3	117	3621.05	4.0973	1.7031	0.1452
Error	16814.5	140	120.10	2.3942		
Total	1195727.4	279				

variance with error. The 5% points for $n_1 = 9$ and $n_2 = 140$, and $n_1 = 13$ and $n_2 = 140$, were obtained by interpolation from Fisher's tables. The 5% point for $n_1 = 117$ and $n_2 = 140$ was calculated by the formula given by Fisher in the section dealing with interpolation. The estimates of variance are all highly significant and there was no obvious need of obtaining the 5% points accurately. In fact the Z values are much higher than the 1% points of the distribution of Z. This can be determined merely by an inspection of the table of 1% points. For $n_1 = 9$ and $n_2 = 140$ we take $n_1 = 8$ and $n_2 = \infty$, 1% point = 0.4604, for $n_1 = 13$ and $n_2 = 140$, we take $n_1 = 12$ and $n_2 = \infty$, 1% point = 0.3908. For $n_1 = 117$ and $n_2 = 140$, the nearest value given in the tables is for $n_1 = 24$ and $n_2 = \infty$ and this value 0.2913 is higher than it should be but our Z value of 1.7031 is very much higher.

The standard error of the experiment is $\sqrt{120.10} = 10.96$ and from this we calculate:

$$\text{Standard error mean of one district} = \frac{10.96}{\sqrt{28}} = 2.07,$$

$$\text{Standard error mean of one variety} = \frac{10.96}{\sqrt{20}} = 2.45.$$

Roughly a difference of $(2.07 \times 3) = 6.21$ cc. between the means of districts is significant and a difference of $(2.45 \times 3) = 7.35$ cc.

between the means of varieties is significant. Again we may, if we wish, present the mean and standard errors on a percentage basis.

The interpretation of this analysis with regard to variety and regional effects on loaf volume is fairly obvious and by means of the standard errors any two districts or varieties can be compared directly. The interaction effect as stated formerly is due to the fact that the varieties did not react in exactly the same manner to soil and climatic conditions. It is difficult to sort out the varieties, if any, in which the differential effect of soil and climate is greatest, but with a knowledge of the agronomic character of the varieties and the nature of the different soils and climates under which they were tested, it might be possible to narrow down the source of the interaction. Without such an analysis we can say only that there is a differential effect and that it is highly significant.

Conclusion

It is hoped that this discussion with examples will enable beginners to obtain a working knowledge of the analysis of variance. In order to obtain a fuller knowledge a study is suggested of the works given below under literature cited. Fisher's book (1931) is indispensable in the use of the analysis of variance. It contains the tables giving the 5% and 1% points of the distribution of Z, and one section of the book is devoted entirely to the application of the analysis of variance to a variety of statistical problems. The mathematical theory involved in the analysis of variance is described by Fisher (1924, 1925), and more recently an especially valuable discussion of the subject has been given by Irwin, (1931). For further details in practical application, the papers by Fisher and Wishart (1930), Wishart (1931), Geddes et al. (1931), and Goulden (1931), will be found very valuabe.

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NON-PROTEIN NITROGEN COMPOUNDS IN CEREALS AND THEIR RELATION TO THE NITROGEN FACTOR FOR PROTEIN IN CEREALS AND BREAD

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Introduction

In a recent circular from the U. S. Department of Agriculture, Jones (1931) has suggested nitrogen factors for use in analyses which, he believes, "will give values representing the real protein content more closely than those obtained by the indiscriminate application of the factor 6.25, now in general use." These factors are offered from his study of the analyses of various proteins as described in the literature. Non-protein nitrogen compounds are mentioned, but no allowance is made for them in the protein factors suggested for use with the total nitrogen found in the various products. These non-protein nitrogen compounds are quite clearly of importance in connection with certain protein factors which are proposed for cereals.

When the writer was connected with the Arkansas Agricultural Experiment Station during the years 1890 to 1899 he made a systematic and extended study of the chemical composition of wheat and its mill-products. This included, among other things, a special study of their nitrogen compounds. In this study a system was devised for the convenient quantitative separation of the different proteins as they had been recently mapped out by Osborne and Voorhees (1893) of Connecticut in their classical researches on the proteins of the wheat kernel. In carrying out these analyses it was found necessary to determine the amount of non-protein nitrogen. By doing this it became possible to check closely the total nitrogen with the sum of that in the several nitrogen compounds present. From these investigations the importance of the non-protein nitrogen compounds in the analysis of these products became apparent. Much of the data given here were published by the author (1896, 1898) in connection with other matter in two bulletins of the Arkansas Experiment Station. As these bulletins were of limited editions, only a few, if any, are now available to students.

The subject matter of this paper is arranged under the following sub-topics: 1. A study of the non-protein nitrogen compounds of

cereals, (*a*) how determined, (*b*) constancy of their presence, (*c*) something of their nature. 2. The selection of protein factors for use in analyses. 3. Cause of variation of factors for individual proteins. 4. The protein factor for bread.

A Study of the Non-Protein Nitrogen Compounds of Cereals

When this early work was done the general method of the Association of Official Agricultural Chemists for determining amido nitrogen in foods and feeding stuffs was, as it now is, to subtract from the total nitrogen the amount of albuminoid nitrogen as found by precipitation with cupric hydroxide. Using this method on wheat it was found that a complete precipitation of the protein substances was not made by that reagent. It was further found that on adding alum, as the method requires for seeds rich in phosphates, the precipitation was less complete than without it. The precipitation also became less complete when the amount of alum was increased.

To overcome this difficulty a method of precipitating the protein by use of phosphotungstic acid was adapted from a method described by Dehérain (1892). This method of separation has since come into extensive use in this country and is official for wheat products. Phosphotungstic acid precipitates proteins from solution, but does not precipitate the non-protein nitrogen compounds in the dilute solutions used.

A step in the method of analysis used was to extract the finely ground material with a 1% solution of common salt. This also extracts the non-protein nitrogen and the presence of the salt is favorable to the complete precipitation by phosphotungstic acid of the proteins in solution. The method as adopted was substantially as follows:

> Five grams of the finely ground material are digested with 250 cc. of a 1% solution of pure sodium chloride in ammonia-free water for two and one-half hours and filtered clear. Ten cubic centimeters of a 10% solution of phosphotungstic acid in 3% hydrochloric acid are added to 100 cc. of the salt solution, mixed intimately and allowed to stand over night. One hundred cubic centimeters of the clear filtrate from this are taken for determination of the nitrogen by the Kjeldahl method.

In a series of mill products made from the medium soft wheat of Northwest Arkansas, the non-protein nitrogen in the air-dry material was found to increase from 0.03% in the short patent flour to 0.23% in the bran. Expressed in per cent of the total nitrogen present, it varied from 1.7% to 14.9%, the latter being on the outer coating of the bran. In a series of spring wheat flours obtained from a then prominent mill in Milwaukee, the non-protein nitrogen in the products ranged from 0.05% in the short patent flour to 0.25% in the low grade or Red Dog. Based on the total nitrogen present, the non-

protein nitrogen ranged from 2.4% in the short patent to 9.4% in the Red Dog. In other tests made in 1912 the non-protein nitrogen in a spring wheat patent flour was 0.03%, in the spring wheat bran 0.22%, and in the wheat germ 0.57%. These quantities expressed in per cent of the total nitrogen present were 1.5% for the flour, 7.0% for the bran, and 10.4% for the germ. The analytical data in this connection are given in Tables I, II, and III.

TABLE I

TOTAL AND NON-PROTEIN NITROGEN[1] IN A SERIES OF ARKANSAS MILL-PRODUCTS, 1896

Kind of product	Total nitrogen	Non-protein nitrogen	Non-protein nitrogen as percentage of total nitrogen
	P.ct.	P.ct.	
Patent flour	1.73	0.03	1.7
Straight flour	1.87	.03	1.6
Low grade flour	2.20	.05	2.3
Ship stuff	2.37	.10	4.2
Bran	2.70	.23	8.5
Sifted dust (outer bran)	1.34	.20	14.9

[1] Air-dry basis.

TABLE II

TOTAL AND NON-PROTEIN NITROGEN[1] IN A SERIES OF PORTER'S SPRING WHEAT FLOUR, SEASON 1896

Kind of product	Total nitrogen	Non-protein nitrogen	Non-protein nitrogen as percentage of total nitrogen
	P.ct.	P.ct.	
Souvenir (extra patent)	2.05	0.05	2.4
OOOO Boss flour (patent)	2.18	.05	2.3
Standard flour (straight)	2.26	.06	2.7
Strong bakers' flour	2.66	.09	3.4
Red Dog	2.66	.25	9.4

[1] Air-dry basis.

TABLE III

TOTAL AND NON-PROTEIN NITROGEN[1] IN SOME SPRING WHEAT MILL-PRODUCTS, 1912

Kind of product	Total nitrogen	Non-protein nitrogen	Non-protein nitrogen as percentage of total nitrogen
	P.ct.	P.ct.	
Minneapolis Family Patent flour 1912	2.29	0.03	1.5
Wheat germ 1912	5.50	.57	10.4
Bran 1912	3.13	.22	7.0

[1] Dry-matter basis.

TABLE IV

TOTAL AND NON-PROTEIN NITROGEN[1] IN CERTAIN WHEATS GROWN IN 1896

Source and/or variety of wheat	Total nitrogen	Non-protein nitrogen	Non-protein nitrogen as percentage of total nitrogen
	P.ct.	P.ct.	
Winter Wheats			
Red wheat, Arkansas	2.48	0.12	4.8
Red wheat, Arkansas (1894)	2.19	.10	4.6
Currell, Kansas	2.64	.09	3.4
Zimmerman, Kansas	2.31	.12	5.2
White wheat, Canada	1.41	.07	5.0
White wheat, Oregon	1.43	.07	4.9
Spring Wheats			
Red wheat, South Dakota	3.36	0.17	5.1
Red Fife, Minnesota	2.16	.09	4.2
Red Fife, North Dakota	1.95	.11	5.6

[1] Air-dry basis.

From the analysis of several wheats obtained from different parts of the country in 1896, the data for which are given in Table IV, the non-protein nitrogen was found to vary from 0.07% to 0.17%; or expressed in per cent of the total nitrogen, the non-protein nitrogen varied from 3.4% to 5.6%.

In some recent determinations made in our laboratories on a limited number of samples of barley, oats, and rye, the non-protein nitrogen was found to vary from 0.10% in the barley to 0.25% with one of the samples of oats. Expressed as per cent of the total nitrogen the range was from 4.1% to 7.2%. The data are shown in Table V.

A few samples of bread were also examined and they showed a non-protein nitrogen content varying from 1.4% to 4.1% calculated as per cent of the total nitrogen present (see Table VI).

TABLE V

TOTAL AND NON-PROTEIN NITROGEN[1] IN SOME OHIO SEED GRAINS, DECEMBER, 1931

Kind of grain	Total nitrogen	Non-protein nitrogen	Non-protein nitrogen as percentage of total nitrogen
	P.ct.	P.ct.	
Oats, Scottish Chief, hulls removed	2.38	0.13	5.5
Oats, Miami, hulls removed	2.26	.14	6.2
Oats, Sixty Day, hulls removed	3.45	.25	7.2
Barley, Velvet, hulls removed	2.44	.10	4.1
Barley, Oderbrucker, hulls removed	2.20	.10	4.5
Rye, Rosen	2.23	.13	5.8
Rye	1.95	.13	6.7

[1] Dry-matter basis.

TABLE VI

TOTAL AND NON-PROTEIN NITROGEN[1] IN SOME WHEAT BREADS, DECEMBER, 1931

Type of bread	Total nitrogen	Non-protein nitrogen	Non-protein nitrogen as percentage of total nitrogen
	P.ct.	P.ct.	
Purchased in open market	2.45	0.100	4.1
Baked in laboratory, no milk or malt	2.06	.033	1.6
Baked in laboratory, no milk or malt	2.36	.033	1.4

[1] Dry-matter basis.

During 1897 and 1898 several series of investigations were carried out on wheat cut daily from first formation of the grain in the head to past maturity. The wheats thus obtained were subjected to the methods mentioned above for the separation of nitrogen compounds. In the tabulated results shown in Table VII the amido nitrogen is

TABLE VII

TOTAL AND NON-PROTEIN NITROGEN[1] FOUND IN TWO SERIES OF WHEATS CUT DAILY FROM FORMATION IN HEAD TO PAST MATURITY AT ARKANSAS AGRICULTURAL EXPERIMENT STATION, CROP YEARS 1897–1898

Period of cutting[2]	1897 series			1898 series		
	Total nitrogen	Non-protein nitrogen	Non-protein nitrogen as percentage of total nitrogen	Total nitrogen	Non-protein nitrogen	Non-protein nitrogen as percentage of total nitrogen
	P.ct.	P.ct.		P.ct.	P.ct.	
1	3.68	0.57	15.5	4.45	2.33	52.4
2	3.25	.28	8.6	3.84	.76	19.8
3	2.85	.21	7.4	3.16	.26	8.2
4	2.63	.18	6.8	2.64	.17	6.4
5	2.55	.16	6.3	2.56	.14	5.5
6	2.36	.11	4.7	2.63	.12	4.6
7	2.45	.10	4.1	2.66	.12	4.5
8	2.69	.10	3.7	2.65	.10	3.8
9	2.78	.09	3.2	2.60	.10	3.8
10	2.95	.10	3.4	2.63	.10	3.8
11	2.91	.09	3.1	2.61	.10	3.8
12	3.02	.11	3.6	2.58	.10	3.9
13	3.03	.12	4.0	2.59	.09	3.5
14	3.17	.12	3.8			

[1] Dry-matter basis.
[2] Numerals in first column represent three-day periods at which samples were taken starting from time of first formation of the grain in the head to past maturity.

shown in three-day periods, each percentage shown being the average of three individual determinations. The non-protein nitrogen present in one of the 1897 series ranged from 0.57% for the first period to 0.09%. These quantities expressed in per cent of the total nitrogen

for those periods were 15.5% and 3.1%, respectively. In a series of tests made in 1898 the total non-protein nitrogen varied from 2.33% to 0.09%, which again expressed in the terms of the total nitrogen is 52.4% and 3.5%, respectively.

In another series of tests, wheat was cut from different plots on succeeding days, and part of the wheat was milled in a small roller mill. The nearly pure endosperm, which had been obtained by careful separation with hand sieves, was analyzed for total nitrogen and non-protein nitrogen. The non-protein nitrogen was found to be 0.02% in each test. The range in per cent of the total nitrogen, however, was from 0.87% to 0.99%. The data for the flour series are shown in Table VIII.

TABLE VIII

TOTAL AND NON-PROTEIN NITROGEN[1] FOUND IN ENDOSPERM OF WHEAT CUT AT DIFFERENT STAGES OF MATURITY

Period of cutting[2]	Total nitrogen	Non-protein nitrogen	Non-protein nitrogen as percentage of total nitrogen
	P.ct.	P.ct.	
6	2.02	0.02	0.99
7	2.08	.02	.96
8	2.27	.02	.88
9	2.26	.02	.88
10	2.31	.02	.87

[1] Dry-matter basis.
[2] Numerals in first column represent three-day periods at which samples were taken.

In a wheat sprouting experiment made in 1895, the results of which have not heretofore been published but are now shown in Table IX,

TABLE IX

TOTAL AND NON-PROTEIN NITROGEN[1] IN WHEAT SPROUTING EXPERIMENT, 1895

Time of sprouting	Total nitrogen	Non-protein nitrogen	Non-protein nitrogen as percentage of total nitrogen
	P.ct.	P.ct.	
Not sprouted	2.66	0.14	5.3
After 24 hours	2.68	.15	5.6
After 48 hours	2.68	.14	5.2
After 72 hours	2.70	.20	7.4
After 96 hours	2.76	.32	11.6
After 120 hours	2.88	.53	18.4
After 144 hours	2.85	.60	21.1

[1] Dry-matter basis.

the amount of non-protein nitrogen was found to increase steadily from 0.14% in the wheat to 0.60% after six days sprouting. In terms

of the total nitrogen present, the non-protein nitrogen varied from 5.2% to 21.1%. Similar data have been recorded by Harcourt (1911), Willard and Swanson (1911, 1913), Fitz and Swanson (1916), Olson (1917), and others.

Since these results are valuable for comparative study, they have been given in detail in the tables. Many other analyses of a similar nature have been made in the course of our work. The results given here are sufficient to show a fair picture of the subject. The presence, nature and distribution of these non-protein nitrogen compounds have a far greater significance than their relation to the usual analyses of food products and should have the careful consideration of students of plant physiology.

It is apparent from the analyses just discussed that all cereals and most cereal products contain some part of their nitrogen in the form of non-protein compounds. The quantities are comparatively large in the newly formed seed and in the germinated seed. There is more in the germ and bran than in the endosperm. For this reason they are more abundant in the lower grades of flour than in the better grades. They are found in all cereals at all times, but the amounts in the same class of material are not wholly constant.

At the time these earlier analyses were made, the non-protein nitrogen was quite generally referred to as amides or amido nitrogen. We now know that it includes the nitrogen of amides, amino acids and certain nitrogen bases and some other compounds such as lecithin. There appears to be no single name that will accurately describe all. The non-protein nitrogen compounds which have been found in wheat are shown in Table X. The composition, amount of nitrogen, and the

TABLE X

NON-PROTEIN NITROGEN COMPOUNDS WHICH HAVE BEEN FOUND IN WHEAT

Compound	Composition	Nitrogen	Nitrogen factor
		P.ct.	
Asparagin	$C_4H_8N_2O_3$	21.21	4.714
Arginin	$C_6H_{14}N_4O_2$	32.18	3.107
Allantoin	$C_4H_6N_4O_3$	35.44	2.822
Betain	$C_5H_{13}NO_2$	10.37	9.643
Cholin	$C_5H_{15}NO_2$	11.57	8.643
Lecithin	$C_{44}H_{84}NPO_9$	1.73	57.8

nitrogen factor, it will be noted, vary for each. The variations in nitrogen and the resulting nitrogen factor, which would give the amount of each pure substance from nitrogen present, show how difficult it would be to use any single factor for calculating from the non-protein nitrogen present the amount of these compounds in a

cereal. The table also illustrates the cause for variations in nitrogen factors for proteins as described in a later paragraph. These data are current information but are shown here for convenience of reference.

Asparagin is an amido compound containing two portions of ammonia. Arginin and allantoin are amino acids of high nitrogen content. Betain and cholin are bases closely related to the alkaloids. Lecithin is of a fatty nature. It is quite certain that none of these, with the exception of lecithin and possible exception of asparagin, furnish any food substance to the animal. One-half of the nitrogen of asparagin is easily split off as ammonia, in which form it would not be useful as food. The asparatic acid which remains is one of the several amino acids of proteins. It contains one-half the nitrogen of the asparagin and this portion of the nitrogen may be useful in foods. Asparagin is present in cereals apparently as a form suitable for moving the protein substances from the leaves and stem to the seed and from the seed to the new plant which grows from it. The purpose of the other non-protein compounds is not so clearly apparent.

Selection of Protein Factors

The method of estimating the protein in foods by calculation from the nitrogen found has been used for a considerable time. An early factor for the purpose was 6.33 (Blyth, 1896; Allen, 1898). This was obtained by assuming that protein of unknown nature had an average of 15.8% nitrogen. Following this, the round number of 16% nitrogen was taken as representative and the factor 6.25 was obtained. No reference was made in either of these to the comparatively small amount of non-protein nitrogen present.

Following the same course of reasoning, the factor 5.7 was selected (Teller, 1896) as a factor for expressing the protein in wheat and its products. This was based upon the average amount of nitrogen found in these proteins by Osborne and Voorhees. It was not looked upon as exact, but was taken as a convenient number close to the truth. The composition of proteins on which it was based was only an average of closely agreeing determinations. Slight variations in the determined amount of nitrogen in one or more of the several proteins would have changed the factor somewhat. There was no reason at that time to take into account the differences due to the non-protein nitrogen, the presence of which was clearly known.

A limited number of preparations and analyses showed the proteins of rye and barley to agree closely with those in wheat, in both composition and character. For this reason it has always seemed that the appropriate factor to use for them was the same as for wheat. The factor 5.7 is close enough to the truth to answer all practical purposes on each of these grains.

Based upon certain determinations on proteins obtained from wheat bran and upon the accepted factor of 5.7 for the proteins of wheat flour, Jones (1931) has calculated a conversion factor of 5.83 as one which "will give the protein content of wheat more accurately than the customary factor 5.7." Jones also states: "The factor 5.83, which has been calculated for wheat, can therefore also be used for rye, barley, and oats."

Following a paper, by the same author, in this journal (1926), the factor 5.83 for wheat was proposed (1927) to the A. O. A. C. as an official factor and has been repeatedly discussed by that association. From this it is apparent that the subject is of more than present interest. Since this proposed factor is based on proteins only, and since the total nitrogen in grains which is found in the usual analysis includes both protein nitrogen and non-protein nitrogen, the presence of the latter should not be overlooked in a search for greater accuracy.

From the analysis shown in the above tables, it is clear that the non-protein nitrogen in whole grain is an appreciable part of the nitrogen present. Let us take as a near average 4.5% of the total nitrogen in mature wheat as the non-protein nitrogen content and 95.5% as the actual protein nitrogen. For example a wheat which has 2.00% total nitrogen will contain 1.91% nitrogen in protein compounds. The remaining 0.09% nitrogen will be made up of non-protein nitrogen compounds. Using the factor 5.83 on the total nitrogen we shall have 2.00% $\times$ 5.83 equalling 11.66% as the apparent protein content. Using the factor 5.7 we would obtain 11.4%. This is a difference of 0.26%. The actual protein nitrogen, 1.91%, multiplied by 5.7 gives us 10.88% as the actual protein. The difference between this and the apparent protein as found by the factor 5.7 is 0.52% or exactly twice the difference between the apparent protein as found by the factors 5.83 and 5.7. At its best, the factor 5.83 does not take us nearer the truth but takes us farther from it.

The amount of non-protein nitrogen in different products is not constant. For most purposes there would be no material gain in determining it and correcting for it in the analysis. The factor 5.7 has, by common usage, come to be the established factor for wheat and its products and has long served a useful purpose as such. In the light of present knowledge is it not better that we should retain it for both grains and flour than to substitute for it some other less convenient factor a little different but giving results that will not be more useful for general purposes and will not be more nearly correct? Many factors for closely related articles are inconvenient and cause confusion.

Cause of Variation of Protein Factors

Within the last thirty years skilled workers have obtained from each of several different proteins nearly a score of distinct compounds, most of which belong to a class known as amino acids. Each contains nitrogen, but the amount of nitrogen differs for each. One has as low as 7.73% nitrogen, and one has as high as 32.0% nitrogen. Besides this, a greater or less amount of ammonia is also separated. This has 82% nitrogen. Of these several products from protein, thirteen contain less than 16% nitrogen. The remainder contain a higher percentage.

The relative proportions of the different amino acids, etc., which have been separated from several proteins vary greatly with the kind of protein. If there is in a protein a relatively large amount of compounds of low nitrogen content and a relatively small amount of compounds of high nitrogen content, that protein will contain a small percentage of nitrogen and will have a relatively high protein factor. The reverse will give a low protein factor. The nitrogen present multiplied by this low factor will show a low percentage of protein, as is illustrated for the non-proteins in Table X.

The present knowledge of the purpose which these different nitrogen compounds serve in the animal body is not sufficient to tell in all cases which protein has the greatest food value. Casein, with its nitrogen factor of 6.38, is considered a more balanced protein from a food standpoint than gliadin with a nitrogen factor of 5.7. Gliadin shows a deficiency which must be supplied by another type of protein. On the other hand, zein, with a nitrogen factor of 6.2, is deficient in at least three amino acids that are considered necessary in foods, including the one which is lacking in gliadin,

The present view is that, in the digestion of a protein in the body, the protein is split up into amino acids quite similar to those that have been produced in the laboratory by chemicals. The body then makes a selection of those amino acids which are necessary to build up its own protein. In this way, in a mixed diet, one protein supplements another. For this reason, if for no other, the rational procedure is to use a variety of foods. In bread the deficiency in the proteins of flour may be made up by the proteins of milk. Milk is extensively used in bread, but probably more particularly for other reasons.

In view of the above facts it is clear that the amount of protein in a food is not fully an index of the protein value of that food. However, it gives valuable information and we use it to good advantage even though we know that it does not tell all.

The Protein Factor for Bread

A recent report by Morrison (1931) of the analyses of several different commercial breads indicates an average of 1.51% nitrogen in the fresh bread, containing 37% moisture. This is equivalent to 2.40% nitrogen in the dry matter.

There are two factors in somewhat general use for calculating from the nitrogen the amount of protein in bread. They are 5.7 and 6.25. Multiplying 1.51 by 5.7 we have 8.61% protein, and by 6.25 we have 9.44% protein. This is a difference of 0.83% on the bread. On the dry basis we have 13.68% protein by use of the factor 5.7, and 15% by use of the factor 6.25. This is a difference of 1.32%. This variation is also reflected in the carbohydrates as determined by difference.

The factor 5.7 is the A. O. A. C. official factor for cereal products. Its use for baked cereal products was emphasized by special action in 1925. This same factor has been incorporated in the accepted methods of the cereal chemists. The factor 6.25 has long been used for mixed foods of unknown composition and unmixed foods of which the composition of the protein has not been determined. Some still use it for calculating the protein in bread, possibly for the reason that some nitrogen-containing ingredients other than flour are used in making the dough or possibly sometimes because its traditional use makes it convenient for increasing somewhat the apparent protein in that food. A method of chemical analyses which needlessly gives an habitual error of near 9% on the amount of the material sought, is hardly acceptable at this time.

In addition to flour, the usual materials used in making bread are yeast, salt, fat or oil, sugar, water or milk, sometimes malt extract and/or complex yeast improvers. Of these, yeast, milk and malt products contain protein. The latter, which is used in small amounts only, has a protein factor essentially the same as flour but there is more amido nitrogen present.

The amount of compressed yeast in the sponge dough now commonly used would be equivalent to about 1½ pounds to 100 pounds of flour. According to many analyses we have made, the crude protein (N × 6.25) in this moist compressed yeast will vary from 12% to 15%, depending on the make of the yeast. Taking the high figure of 15%, we would add to the flour approximately 0.22 pound of crude protein. A series of determinations made many years ago showed that at least ⅛th of this crude protein is non-protein nitrogen substance. From this we reason that the factor for yeast to give the true protein added should be distinctly less than 6.25—even less than 5.7. The usual amount of dry skim milk used by commercial bakers

at the present time is probably about 3 pounds to 100 pounds of flour. This will contain about 1 pound of milk protein (N × 6.38). If sweetened condensed milk were used, the amount of protein added would quite certainly be distinctly less. Shall we take for our present illustration an added 1 pound of milk protein? Let us assume that the flour used contained 11.4% protein. We shall then have in the dough a total of 11.4 + 1.0 + .2 or 12.6 pounds of protein. For the factors used we shall have of nitrogen a total of 2.00 + .16 + .03 = 2.19%. Using the factor 6.25 for the bread, we shall have an apparent protein of 13.7 pounds. In other words, we shall apparently have produced 1.1 pounds of protein in the simple making of bread from 100 pounds of flour. It is hardly possible that any student of this subject has a serious thought that this process does or can materially change the composition or amount of protein of the ingredients used in making the dough. Where, then, do we get the increase?

We will, for this discussion, disregard the small amount of non-protein nitrogen present in milk and yeast. From the data given earlier in this article, we may justly assume that 2% of the total nitrogen in the flour used was in non-protein nitrogen compounds. On the basis of the 2.0% of nitrogen in the flour only 1.96% is nitrogen belonging to actual protein (1.96 × 5.7 = 11.17). This lessens the actual protein 0.23% or more than double the calculated 0.1% which we have gained because of the higher protein factor for the milk used. In other words, the factor 5.7 will give close to the true amount of protein in the bread even when 6% of dry skim milk is added to the flour as is now sometimes recommended.

Because of the complex nature of cereals and cereal products, we cannot always get mathematical exactness in these analyses. All we can hope for is a close approximation. It is to our interest and the interest of those we serve to get that approximation in the simplest form possible. In the case of bread we have a varying product. Sometimes it is made of plain flour, sometimes with varying quantities of other ingredients. Some of these may contain protein of a composition which might make some slight difference in the calculated protein of the bread. We cannot vary the factor to meet all changes with theoretical exactness, for they are generally not known to us. The factor we use for flour meets the conditions as fully as any we may hope to get. In general it gives results which are beyond question for almost any use to which they may be applied.

Summary and Conclusions

During the last half century great progress has been made in our knowledge of the kind and composition of the nitrogen compounds of

various foods. With greater knowledge of the proteins there have been modifications of the factors used to multiply the nitrogen into the corresponding proteins. Heretofore little attention has been given to the non-protein compounds in cereals and their relation to the actual protein present. With attempts to apply a better knowledge of the composition of these proteins to a greater refinement of the protein factors, the non-protein nitrogen becomes a matter of importance.

It is the purpose of this paper to direct the attention of cereal chemists to this point that they may see its bearing on the problem and avoid a useless increase in the number of factors. The protein in cereals and cereal products is becoming of much importance in commercial enterprises. Many factors on the same class of foods serve only to confuse. They lessen the value of the work the chemist is doing.

The factor 6.25 for protein in bread is not in accord with facts and does not give results suited for use in solving many problems in which it has a part. Articles other than flour used in bread do not, in the amounts used, materially increase the protein factor over that of the flour, and there is nothing in the process of bread making that changes the protein factor for the nitrogen of the flour used.

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A TECHNICAL PAPER

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The intention in publishing this new periodical is for reporting work and advances in the milling and baking industries; to give a short resumé of new ideas and problems and to serve as a liaison member between science and the industries concerned without entering to any great extent into the field of scientific publications to duplicate the material which they publish. It is the publisher's endeavor to portray internationally new advances in and modern knowledge of the sciences involved in the milling and baking industries.

An idea of the scope of the publication may be obtained from the following titles of the articles appearing in Volume 1, No. 1, January–February, 1932 (8 pages):

Is the Baking Test a Measure of Flour Value?
An American Letter (A Note on Flour Quality in Relation to Bread Baking Costs.)
Ash Content and Flour Yield.
Concerning Spark Gaps.
From the Mixer (News Notes).
Fifty-five Years of Flour Moths.

The paper is to be free from an advertising section and devoted entirely to short discussions of the nature indicated.

GEO. E. HOLM.

PHYSICAL AND CHEMICAL PROPERTIES OF ETHER-SOLUBLE CONSTITUENTS OF WHEAT FLOUR IN RELATION TO BAKING QUALITY [1]

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Introduction

Baking quality in wheat flour is determined very largely by the quantity and quality of the gluten proteins which it contains. The *quantity* factor is satisfactorily evaluated by practical laboratory methods which are in general use in the wheat and flour trade. The quality factor, however, is less clearly understood. In its simplest aspects, quality may be defined as that property of the gluten which is responsible for gas-retention in the dough. Dough is usually considered to be composed of an interlacing network of gluten strands or fibrils enmeshing the starch and other constituents. During fermentation, bubbles of carbon dioxide gas are formed throughout the dough mass, and when the dough has risen ready for the oven, it becomes a stable extensible foam. Upon baking, the starch undergoes gelation while at the same time the proteins are heat-coagulated, thus transforming the foam into bread. If an insufficient quantity of gluten is present, or its tensile strength is not sufficient to support the pressure of the gas-bubbles, the latter coalesce to form large bubbles, resulting in bread of coarse texture. The *quality* factor is thus definitely associated with the tensile strength of the gluten.

The tenacity of the dough is obviously determined by the chemical constitution and by the physico-chemical environment of the gluten proteins. Although the former is most commonly associated with tensile strength in gas-retention, chemical studies (Blish, 1916) have failed to reveal any significant differences in the proteins of strong and weak flours. It is well known, however, that the properties of the gluten are very markedly influenced by the presence of substances, dispersed in the dough. Gortner (1918, 1923, 1924) and Sharp (1922, 1923, 1924) and their co-workers, Johnson (1930), and others, have shown that the imbibitional capacity of the gluten proteins is greatly influenced by the presence of electrolytes. The latter may exert their

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effects either through chemical combination or through adsorption, the degree and the nature of the effect being determined by the concentration and chemical properties of the electrolytes present. The complex nature of the proteins, and their extreme lability render them highly sensitive to the influence of environmental forces. In the bread dough their properties are determined very largely by the substances bounding their interfaces.

Working (1924) expressed the view that the lipoids normally present in flour influence the physical properties of gluten very markedly. He has shown that prolonged washing of gluten from low grade flour removed phosphatide and gradually increased the tenacity of the gluten until it was practically equal to that from a patent flour. Working (1928) also found that when phosphatide was added to flour in small quantities, the *quality* of its gluten was injured, as indicated by the feel of the washed gluten, viscosity data, and by laboratory baking tests. Johnson (1928), and Johnson and Whitcomb (1931), found that the removal of the ether-soluble constituents of flour from the hard red spring wheats improved their baking qualities. Geddes (1930) studied the influence of the germ constituents on baking quality and obtained like results. Johnson and Whitcomb found that the addition of lard to ether-extracted flour impaired the gas-retaining power of the resulting dough. According to Mohs (1924), the naturally occurring fat-like materials in the dough are in a highly dispersed condition and are so effectively adsorbed by the gluten-forming proteins that they can not be removed by washing. Mohs concluded that these ether-soluble substances were necessary to the formation of gluten, in that they were associated with the hydration of the proteins and consequently rendered the latter more elastic. However, Johnson (1928), and Johnson and Whitcomb (1931), showed quite conclusively that the gas-retaining power of doughs made from ether-extracted flours was significantly higher than it was for those from un-extracted flours. It would appear that the removal of the adsorbed fat-like material increased the tensile strength of the gluten fibrils. Obviously, the quantity and the chemical nature of these adsorbed ether-soluble materials must be an important factor in determining gluten quality in flour.

The Problem

The fact that there are apparently no chemical differences in the gluten proteins from strong and weak flours, and that their physical properties are very profoundly influenced by the presence of surface-active substances of a fatty nature, suggests the possibility of *quality* being an expression of the colloidal behavior of the gluten in the dough, rather than of the chemical constitution of the proteins themselves.

To test the validity of this hypothesis, a comparative study has been made of the chemical and physical properties of the fat-like substances extracted from flours whose gluten proteins were known to differ widely in quality. Flours from Marquis and Kubanka wheats were chosen for these studies because they are both high in gluten but differ greatly in baking quality. Flour milled from Federation, a soft wheat, was also studied. The superior baking quality of flour from the hard red spring wheats, of which Marquis is typical, is generally associated with the quantity and the quality of the gluten proteins which they contain. Flour from Kubanka, a high protein durum wheat used extensively in the manufacture of macaroni and spaghetti, however, is unsatisfactory for making yeast-leavened bread, on account of its gluten not possessing sufficient tensile strength for gas-retention in the dough. As pointed out by Norton (1906), Mangels (1926), and Vogel and Bailey (1927), the average protein content of the durum wheats is slightly higher than that of the common or vulgare wheats. Since the protein content of these wheats was about equal, Mangels concludes, that "The relatively low baking quality of the durum wheats is evidently due to poor quality of gluten." Vogel and Bailey also found that the glutenin-protein ratio for the durum flours was about the same as that for the vulgare wheats; also that there was no significant difference in the glutenin of these flours as indicated by the quality constant b determined according to the method of Gortner (1924).

While it has been inferred that *quality* in gluten as defined at the outset, is more apt to be associated with the physical state of the gluten proteins than with chemical constitution, this must yet be regarded as an open question in the light of the data presented by a number of workers. Woodman (1922), for example, in his racemization studies on the gluten proteins of a strong flour milled from a hard Manitoba wheat and of a weak flour from a soft English wheat, concluded that a strong wheat synthesizes one type of glutenin, and the weak wheat another; while wheats of intermediate strength may synthesize varying proportions of the two glutenins. The differences in the specific rotation values for the racemized glutenins isolated from these two wheats are quite striking. Kent-Jones (1927) repeated Woodman's work and obtained results which, although not so striking as the latter's, were in agreement. However, Kent-Jones felt less enthusiastic about the racemization method as a means of differentiating between the glutenin from strong and weak flours, and concluded that the slight differences observed could not account for the marked differences in the colloidal behavior of the gluten in the doughs when baked into bread. Blish and Pinckney (1924) applied similar methods

to flours from a number of American wheats and found significant differences in the specific rotation of the racemized gliadin, but their results for glutenin did not confirm those of Woodman. They finally concluded, however, that the variations in the flour strengths of the commercially important American wheats could not be attributed to differences in the configuration of their respective glutenin molecules. From a consideration of the probable errors involved in the racemization and polarimetric measurements, Kent-Jones agrees with Blish and Pinckney in concluding that "It is highly improbable that variations in the strength of commercial flours can be looked for in this way." Wood (1907) studied the gluten proteins from flours which varied in baking strength and concluded that they differed only in physical properties. Blish (1916) in his studies on the hydrolytic products of the gluten proteins from weak and strong flours found that they were identical in their chemical constitution as determined by Van Slyke analysis. Similar results were obtained by Cross and Swain (1924) whose data showed that the glutenin, and also the gliadin from wheat flours, while differing widely in baking quality, were chemically identical.

Most of the experimental data presented in the literature support the conclusion that there is no significant difference in the chemical constitution of the gluten proteins from strong and weak flours; and that baking quality, in so far as it is affected by gluten *quality*, is determined by the colloidal behavior of the gluten proteins in the dough. The present work was undertaken, therefore, with the object of throwing some additional light on the question concerning the quantity and physical and chemical nature of the fat-like substances associated with flours whose gluten proteins were known to differ widely in *quality;* and to determine the effect of removing these constituents on the baking strengths of the flours concerned.

Experimental

MATERIALS: Patent flours were produced in an experimental mill from Marquis, Kubanka, and Federation wheats. In order to determine the influence of the removal of the ether-soluble constituents on gluten *quality*, as shown by the baking test, the *quantity* factor was eliminated, as far as possible, by reducing the protein content of the flours from Marquis and Kubanka to approximately the same value as that of the Federation by the addition of ether-extracted starch. The flours used in the un-extracted and ether-extracted studies were not milled at the same time, but the same procedure was followed with each sample, hence they were comparable within the error of experimental milling practice.

EXTRACTION OF ETHER-SOLUBLE CONSTITUENTS FROM FLOUR: Samples of about one kilogram of flour were placed in small two-ply closely woven cotton cylindrical bags in a Lloyd extractor and extracted with anhydrous diethyl ether for 72 hours. About 2 litres of ether were used to extract each kilogram of flour. The usual oven-drying procedure before extraction was omitted in order to avoid, as far as possible, the effects of heating on the constituents extracted.

LABORATORY BAKING TEST: The basic formula of the standard experimental baking test, Blish (1928), was used, and all determinations carried out in duplicate. Tests were made on the un-extracted and ether-extracted flours, as well as upon the flours which had been brought to approximately the same protein content by the addition of ether-extracted starch.

ESTIMATION OF GLUTEN: The gluten was washed under controlled conditions at 30° C., care being taken to wash all samples to a uniform degree. The determinations were made in duplicate. The washed gluten was puffed in an oven at 180° C. and dried to constant weight at 100° C.

Properties of Ether-Soluble Constituents

QUANTITATIVE ESTIMATION: After completing the extraction as described in the foregoing section, ether was distilled from the extract until its volume was reduced to 200 cc. The extract was then filtered and warmed on a water bath at 45° C. with occasional shaking until it was entirely free from ether. This required about 4 hours as determined by reweighing at one-hour intervals.

SPECIFIC GRAVITY: A Nicol specific gravity tube was used and the determination was carried out at 25° C.

COLOR: The relative color of the oils was evaluated by means of a Klett colorimeter. A solution containing 0.005 gms. of potassium dichromate in 100 cc. was used as a standard, and its color value taken as 1.0.

VISCOSITY: An Ostwald type of viscosimeter was used and the coefficient of viscosity calculated from the time-density data. The measurements were made at 25° C.

SURFACE TENSION: Measurements were made at 25° C., using a du Noüy tensiometer.

SOLUTION TEMPERATURE OF SOLIDS SUSPENDED IN OIL: The oil was drawn into a capillary tube, immersed in a water bath, and the temperature at which the suspended solids became translucent taken as the solution temperature. It was necessary to cool the oil from Kubanka wheat flour to below − 16° C. to bring about the formation of solid particles.

INDEX OF REFRACTION: The refractive index was determined at 25° C. with an Abbe refractometer.

OPTICAL ROTATION: The angular rotation in degrees was obtained by the use of a Soleil-Ventzke saccharimeter, using a conversion factor.

FREEZING-POINT DEPRESSION OF 1.0 GRAM OF THE OIL IN 10.0 CC. BENZENE: The depression of the freezing-point of a solution of 1.0 gm. of oil in 10 cc. benzene was determined by the Beckman method, a correction being made for under-cooling by use of the formula given by Gortner (1929) for aqueous systems, but modified for benzene solutions as follows:

$\Delta = \Delta' - 0.032689\, u\Delta'$, where Δ = the corrected depression of the freezing-point; Δ' = the observed depression of the freezing-point; u = under-cooling in degrees Centigrade before separation of crystals begins. The numerical constant, 0.032689 is derived from the latent heat of fusion for benzene which was taken as 30.6 calories per gram.

APPARENT MOLECULAR WEIGHT: Relative values were calculated from the freezing-point data, using the expression $M = (kw)/(dW)$, where M = apparent molecular weight, k = molar freezing-point constant of solvent $\times$ 1000 (for benzene, $k = 5.12 \times 1000$), w = grams of substance (oil), d = difference in freezing-point of solvent due to added substance, and W = weight of solvent in grams.

SAPONIFICATION NUMBER: The A. O. A. C. (1925) official method was used and the results expressed as the Koettstorfer number.

IODINE NUMBER: The iodine number was determined by Wij's method in accordance with A. O. A. C. (1925) procedure.

ACETYL NUMBER: The method recommended by Holland, et al. (1915) was followed, and the difference in the saponification values of the fat before and after acetylation expressed as the acetyl value.

REICHERT-MEISSL NUMBER: The A. O. A. C. (1925) official method was used.

WATER-SOLUBLE ACIDS: The water-soluble acids were determined in accordance with the A. O. A. C. (1925) official method and the results expressed as equivalents per kilogram of fat instead of in terms of butyric acid.

WATER-INSOLUBLE ACIDS: The A. O. A. C. (1925) official method was used and the results expressed as equivalents per kilogram of fat.

NITROGEN: Five-gram portions of the oil were digested by the Kjeldahl method. It was found that an abnormal quantity of sulphuric acid was consumed in oxidizing the oil and consequently more acid was added from time to time during the digestion. The ammonia was distilled off and titrated in the conventional way.

PHOSPHORUS: Five-gram portions of the oil were digested with concentrated nitric acid on a steam plate over a period of 72 hours, after

TABLE I

EFFECT OF ETHER-EXTRACTION ON THE BAKING QUALITY OF FLOURS OF DIFFERENT PROTEIN CONTENTS: AND ON FLOURS BROUGHT TO APPROXIMATELY THE SAME PROTEIN CONTENT BY THE ADDITION OF ETHER-EXTRACTED STARCH

Sample	Protein content[1]	Ash[1]	Gluten[1]	Absorption	Standard laboratory baking test: Weight of loaf	Volume of loaf	Color	Texture
	P.ct.	P.ct.	P.ct.	P.ct.	Gms.	Cc.	Score	Score
Wheat								
Marquis		1.61						
Kubanka		1.50						
Federation		1.02						
Untreated flour								
Marquis	14.6	0.40	17.9	68	129	458	98	105
Kubanka	14.2	0.61	17.5	69	127	440	75	95
Federation	10.3	0.46	11.6	62	127	400	65	75
Ether-extracted flour								
Marquis	14.8	0.42	17.2	69	129	463	105	110
Kubanka	14.6	0.68	15.2	71	126	363	90	98
Federation	10.5	0.50	10.4	60	123	310	85	40
Untreated flour, with added starch[2]								
Marquis (59.2 gm. flour, 25.8 gm. starch)	10.5			70	136	360	99	102
Kubanka (60.6 gm. flour, 24.4 gm. starch)	11.0			73	133	383	80	85
Federation (85.0 gm. flour, 0.0 gm. starch)	10.3			66	137	390	70	75
Ether-extracted flour, with added starch[2]								
Marquis (58.7 gm. flour, 26.3 gm. starch)	10.4			76	132	383	105	104
Kubanka (60.3 gm. flour, 24.7 gm. starch)	10.3			67	127	360	100	90
Federation (83.6 gm. flour, 1.4 gm. starch)	10.4			67	123	373	85	30

[1] All values are given on the moisture-free basis.

[2] Sufficient ether-extracted corn starch was added to reduce the protein content to approximately 10.5%. The starch contained 7.7% of moisture and its nitrogen content was equivalent to 0.55% of protein.

TABLE II

PROTEIN CONTENT OF WASHED GLUTEN FROM UNTREATED AND ETHER-EXTRACTED FLOURS; ALL VALUES GIVEN ON MOISTURE-FREE BASIS

Sample	Untreated flour: Washed gluten	Untreated flour: Protein content of washed gluten (N×5.7)	Ether-extracted flour: Washed gluten	Ether-extracted flour: Protein content of washed gluten (N×5.7)
	P.ct.	P.ct.	P.ct.	P.ct.
Marquis	17.90	72.10	17.00	74.80
	18.00	74.10	17.40	72.10
Average	17.95	73.10	17.20	73.85
Kubanka	17.70	74.20	15.50	80.00
	17.30	74.80	14.90	82.20
Average	17.50	74.50	15.20	81.10
Federation	12.10	73.00	9.80	79.80
	11.10	75.80	10.90	76.30
Average	11.60	74.40	10.35	78.05

which magnesium nitrate was added to complete the oxidation. The phosphorus content of the ash was then determined gravimetrically.

Discussion

EFFECT OF ETHER-EXTRACTION ON BAKING QUALITY: The results of the flour studies and baking tests, and the values for the protein content of the washed gluten are presented in Tables I and II respectively; and the cross-sectional views of the experimental test loaves baked from the various flours are shown in Figure 1. The effect of ether-extraction on the color, texture and volume of the bread is quite apparent in the latter. As shown in Table I, ether-extraction resulted in a slight increase in the loaf-volume for Marquis, and in a marked decrease in that for both Kubanka and Federation. Removal of the ether-soluble constituents from the flour improved the color of the bread from all three of the wheats, while it improved the texture of that from Marquis and Kubanka and had a very noticeable deleterious effect on the texture of the bread from Federation flour.

The effect of ether-extraction on the baking quality of the flour from Marquis wheat agrees well with the results reported by Johnson and Whitcomb (1931) for high and low grade flours from hard red spring wheats, milled on a long-system mill. These results are also in agreement with those reported by Geddes (1930) for straight grade flour. The removal of the ether-soluble constituents from the Kubanka wheat flour apparently affected the production of carbon dioxide in the fermenting dough with a consequent decrease in loaf volume. That the latter was caused by the production of insufficient carbon dioxide gas, and not by the poor gas-retaining properties of the dough, is indicated by the formation of smaller bubbles which resulted in a finer texture in the bread. If the lower loaf-volume had been caused by a loss of carbon-dioxide from the dough due to low tensile strength in the latter, the gas bubbles formed in the fermentation process would have coalesced to form larger bubbles, resulting in a bread of coarse texture. An improvement in the texture with an accompanying decrease in loaf-volume was observed for the ether-extracted Kubanka flour both with and without the addition of starch, which seemed to indicate very definitely that the effect on baking quality was associated with gas-production and not with gas-retention in the dough. It would thus appear that the nutrition of the yeast cells was in some way affected by the removal of the ether-soluble constituents from Kubanka flour.

The deleterious effect of ether-extraction on the Federation wheat flour, as indicated by poor texture and low loaf-volume of the resulting bread, is difficult to explain, in view of the results obtained with

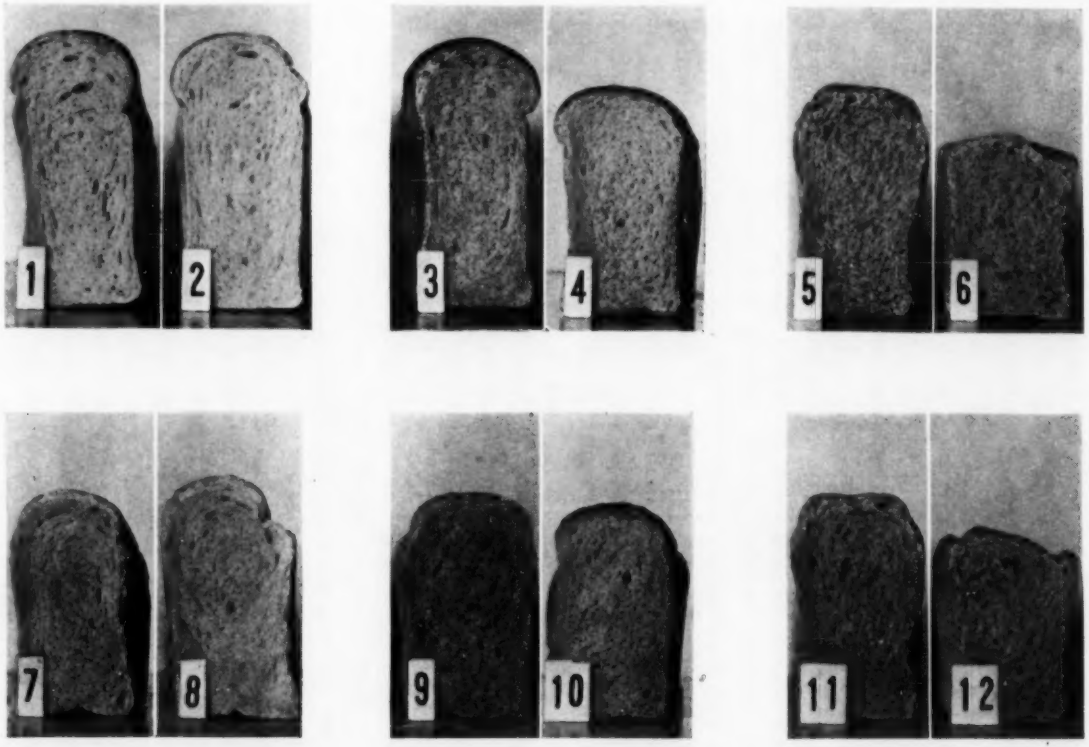

Fig. 1. Photographs of loaves baked from natural and ether-extracted flours.

Upper row—(1) Marquis, untreated; (2) Marquis, ether-extracted; (3) Kubanka, untreated; (4) Kubanka, ether-extracted; (5) Federation, untreated; (6) Federation, ether-extracted.

Lower row—(7) Marquis, untreated with added starch; (8) Marquis, ether-extracted with added starch; (9) Kubanka, untreated with added starch; (10) Kubanka, ether-extracted with added starch; (11) Federation, untreated with added starch; (12) Federation, ether-extracted with added starch.

Marquis flour which had been brought to approximately the same protein content as that of Federation by the addition of starch. The coarser texture and lower loaf-volume of the bread indicated quite definitely that the extraction of the ether-soluble constituents from the Federation flour impaired the gas-retaining properties of the dough. This is just the reverse of the effect observed with Marquis wheat flour, and of the results obtained by Johnson and Whitcomb (1931) and Geddes (1930) for flours from Marquis, and other hard spring wheats. The data obtained in this investigation show that ether-extraction does not affect in the same way the baking quality of flours from widely different wheats, and that *quality* in gluten is affected by a number of factors.

As shown in Table II, the protein content of the washed gluten was appreciably higher for the ether-extracted than for the un-extracted flours; the differences being 0.75%, 6.60%, and 3.65% for Marquis, Kubanka, and Federation wheats, respectively. These differences are accounted for in part by the removal of more of the non-protein material from the extracted than from the un-extracted flours in the gluten-washing process. The differences in the washed gluten from extracted and un-extracted flours from Marquis, Kubanka, and Federation were 0.75%, 2.30%, and 1.25%, respectively. These results are not in agreement with those for hard red spring wheat flours, milled on a long-system mill, reported by Johnson and Whitcomb (1931), who stated that: "Satisfactory glutens were obtained from both un-extracted and extracted flours and the quantity of gluten (wet or dry) was the same for the extracted as for the corresponding natural flour." Again, these studies show that flours from widely different wheats may be expected to respond differently to ether-extraction.

PROPERTIES OF ETHER-SOLUBLE CONSTITUENTS OF FLOUR IN RELATION TO BAKING QUALITY: The data in Table III show that the ether-soluble constituents extracted from the Marquis, Kubanka, and Federation flours differed quite widely in some of their physical and chemical properties. The flour from Kubanka contained appreciably more ether-soluble matter than did that from either Marquis or Federation wheats. These results parallel those of Sullivan and Near (1927), who found that the durum wheats were higher in lipoids than were the vulgare wheats.

The properties of the oils extracted from the three wheats which differed most markedly were color, viscosity, total acids, water-soluble acids, and the amount of crystalline solids in suspension. The high content of carotinoid pigments in the Kubanka flour was reflected in the high color value of its oil. The low viscosity of the latter seemed

to indicate that the ether used in extracting it from the flour had not been completely removed, and accordingly the sample was heated to 45° C. on a water bath for an additional four-hour period, but this did not change appreciably the initial viscosity value.

TABLE III

PHYSICAL AND CHEMICAL PROPERTIES OF ETHER-SOLUBLE CONSTITUENTS EXTRACTED FROM MARQUIS, KUBANKA AND FEDERATION WHEAT FLOURS

Properties of ether-soluble constituents	Marquis	Kubanka	Federation
Non-volatile ether-soluble constituents extracted from 1 kg. flour (grams)	13.29	14.87	11.71
Color value (the color of a solution of 0.005 gm. $K_2Cr_2O_7$ in 100 cc. taken as 1.0)	7.0	61.0	33.0
Coefficient of viscosity	1.47	0.49	1.42
Specific gravity	0.9399	0.9251	0.9418
Surface tension (dynes per cm.)	36.57	35.91	36.68
Solution-temperature of suspended solids (°C.[1])	45 to 49	−16 to −18	41 to 45
Index of refraction (angular degrees)	1.4805	1.4785	1.4798
Optical rotation (angular degrees)	0.69	0.48	0.55
Freezing-point depression of 1.0 gm. in 10 cc. benzene (° C.)	0.838	0.851	0.618
Apparent molecular weight	695	689	942
Saponification number (Koettstorfer number)	182.2	177.7	183.4
Iodine number	121.9	127.6	126.8
Acetyl number	21.8	18.4	24.2
Reichert-Meissl number	1.50	1.56	1.06
Total acids (equivalents per kg. of oil)	2.7744	2.9037	2.6916
Water-soluble acids (equivalents per kg. of oil)	0.2536	0.3671	0.1868
Water-soluble acids (equivalents %)	9.1	12.6	6.9
Water-insoluble acids (equivalents per kg. of oil)	2.5208	2.5366	2.5048
Water-insoluble acids (equivalents %)	90.9	87.4	93.1
Nitrogen %	0.57	0.61	0.30
Phosphorus %	0.15	0.16	0.14

[1] Temperature at which suspended crystals (sterols) dissolved in oil.

The property which most strikingly differentiated the Kubanka oil from that of the other two wheats was its freedom from suspended crystalline solids at ordinary temperatures. The oils extracted from both Marquis and Federation contained appreciable quantities of small crystals in suspension similar to those observed by Gortner (1908) and later identified by Ball (1926) as a sitosterol ester. Anderson, Shriner, and Burr (1926) in their studies on wheat germ oil concluded that the sterols of the germ oil were composed of a mixture of dihydrositosterol and at least three sterols isomeric with sitosterol which they designated as α-, β- and γ-sitosterol. The melting-point of these sterols was in the neighborhood of 135° to 145° C. As given in Table III, the crystals suspended in the oils from Marquis and Federation disappeared (visibly at least) when the temperature of the oil was raised to 41° to 49° C. This is not considered the melting-point, but

rather the temperature at which the crystals dissolved in the oil. The Kubanka oil was perfectly clear until the temperature was lowered to − 16° C., when small plate-like crystals appeared. Whether these were identical with those in the Marquis and Federation oils at room temperatures was not determined. However, these observations indicate that the oil from the vulgare wheats contained appreciably more sterols than did that from the durum wheat. Similar results were reported by Walde and Mangels (1930) for acetone extracts from vulgare and durum wheats. The latter authors suggest that the presence or absence of certain forms of sterols may have an important effect on gluten quality.

The water-soluble acid content of the oils extracted from the three flours differed significantly; the values for Marquis, Kubanka, and Federation being 0.25, 0.37, and 0.19 equivalents of acid per kilogram of oil respectively. As shown in Table III, the total acid content of the oil was correspondingly higher for Kubanka than for that of the other two wheats, indicating that the difference was largely in the amount of the water-soluble acids.

Notwithstanding the significant differences in the properties of the oil extracted from the three flours, the different effects of ether-extraction on baking quality of the flours, when brought to approximately the same protein content by the addition of starch, indicate that they probably contained gluten proteins differing in chemical constitution. *Quality* in the gluten proteins would thus appear to be determined primarily by chemical constitution, and secondarily by physico-chemical environment.

Summary

Some of the physical and chemical properties of the ether-soluble constituents extracted from Marquis, Kubanka, and Federation wheat flours have been determined. The effect of removing these substances on the baking quality of the flour has also been investigated. The results obtained may be briefly summarized as follows:

1. Ether-extraction improved the baking quality of the flour from Marquis, but had a deleterious effect on that from both Kubanka and Federation wheats. A marked improvement was noted in the color of all three of the extracted flours. The loaf-volume and texture data show that ether-extraction of the flour improved the gas-retaining power of the dough from Marquis, but that it impaired the gas-producing, and not the gas-retaining, power of the dough from Kubanka wheat flour. The fermentation process was apparently affected by the removal of the ether-soluble constituents from the Kubanka wheat flour. The decrease in both the loaf-volume and the

texture score for the bread from Federation wheat indicates that ether-extraction of the flour impaired the tensile strength and, incidentally, the gas-retaining power of the resulting dough.

2. Significant differences were observed in the properties of the oils extracted from the different flours, notably color, viscosity, water-soluble acids and sterols. However, the different effects of ether-extraction on the baking quality of the bread from the three flours indicate that gluten *quality* is probably determined more by differences in the chemical constitution of the gluten proteins and their physical-chemical environment than by the presence or absence of fat-like substances.

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SOME OBSERVATIONS ON NUTRITION [1]

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Let us start our observations with three brief quotations. The first comes from Sir William Osler:

"There is not one thing in preventive medicine that equals mouth hygiene and the preservation of the teeth."

The second is due to Mrs. May Mellanby:

"Of all the disorders which beset humanity it is probable that dental diseases are responsible, directly or indirectly, for a larger aggregate of ill health and unhappiness than any other form of disease."

The third and last quotation comes from Dr. McKim Marriott. To appreciate Dr. Marriott's statement, it should be contrasted with the old and familiar slogan that "A clean tooth never decays." Dr. Marriott (1929) has modified this old slogan into a more modern form:

"A well-fed tooth never decays."

Any who have been so unfortunate as to suffer the pangs of toothache, not infrequently incident to dental caries, will doubtless agree with Bobby Burns when he calls it the "Hell of a' diseases."

Since the old slogan has been given you that "a clean tooth never decays," let us have another old one—old but still modern—

"A tooth for every child."

There is something fundamentally wrong when Nature takes this extra toll from the mother. Yet so common is dental caries in all walks of life that I veritably believe that many people consider it to be the normal order of events—perhaps as natural, as Job said, as it is for sparks to fly upward.

Dr. Cross (1923), formerly of the Forsyth Dental Infirmary of Boston, has stated:

". . . 96% of the children coming to this country from the southern part of Europe have sound teeth, while it is well known that the teeth of 96% of the American children are defective. Strange to say, too, that the vast majority of the foreign children never knew what a toothbrush looked like until they entered the American schools."

[1] A lecture delivered before the New York and the Midwest Sections of the A. A. C. C. Since the lecture was illustrated with lantern slides, the text here published has been modified to cover their omission, as well as to include a brief mention of a few points which came up for subsequent discussion.

It would be bad enough if dental caries ended merely with the loss of the teeth involved, but so often it is the source of highly undesirable systemic infections.

Let us go back about 50 years. In the early eighties of the last century, Dr. W. D. Miller—an American dentist—was working with Dr. Robert Koch, the famous German bacteriologist. Miller developed the idea that dental caries was basically caused by bacteria, or the products of their activities. The bacterial origin of this disease was accepted and has ever since been believed in, although with the newer knowledge of nutrition, we now have evidence that there is a nutritional factor involved. Saliva contains mucin, a substance which settles on the teeth in the form of a film or placque. That these placques are not figments of our imagination can be readily determined, for they are readily stained with Bismarck Brown or iodine, neither of which stains clean enamel. Since the oral cavity is never free from bacteria, these placques naturally contain some of them.

These bacteria, especially those of an acid-forming character, convert some of the carbohydrate material of our food into lactic acid, which acid, being held against the enamel, begins to etch it and eventually to such an extent that we have an invasion, which we call decay or dental caries. In this process, certain proteolytic enzymes, produced by bacteria, are also supposedly involved.

Now in opposition to this picture, there are mouths in which the teeth are completely covered with these germ-laden placques—mouths in which there has been absolutely no attempt at oral cleanliness—yet the teeth are sound. The answer seems to be but one thing, namely, that the tooth substance has developed a resistance to bacterial invasion, has set up a species of immunity, as we might say. It would therefore appear that there are two factors involved in this problem of dental caries—one bacterial, the other that of tooth resistance. So we must look to doing what we can to build up the resistance of the teeth, for we can never hope to keep the mouth sterile.

If food is a primary factor, then we should reasonably expect a reflection of this fact either in the tooth structure or in its chemical composition, as has been suggested by Howe (1926), or possibly in both. The subject of the structure has been extensively studied by Mrs. May Mellanby (1928), of England, and she concludes—

> " that there is a definite relation between defective dental structure and caries, and that the structure of the teeth can be controlled by diet."

While her experimental work has been done largely with dogs, it has been extended so as to include rabbits, rats, and, on a smaller scale, human beings,

> " the results being in all cases clearly comparable to those previously found with dogs."

Mrs. Mellanby's husband—Prof. E. Mellanby—has also been a notable worker in this field. According to him—and this is something of particular interest to us as cereal chemists—certain cereals, notably oatmeal, tend to prevent calcification of teeth and he suggests the name "toxamin" for this anticalcifying substance (Mellanby, M., 1929). As far back as 1922, he held that vitamin D was the central factor in calcification.

In her experiments, Mrs. Mellanby fed puppies on identical diets of separated milk powder, lean meat, orange juice, yeast, sodium chloride, cereal and fat, the last two being the variables. She found that when the cereal was white flour and the fat, linseed oil, the enamel was fairly thin, as was also the dentine, the latter containing interglobular spaces. If, in addition to the white flour and the linseed oil, the diet contained some commercial germ, the results on the tooth structure were worse in every way, but not as bad as when the cereal was oatmeal and the fat, linseed oil. On the other hand, if the diet contained oatmeal and the linseed oil was replaced by cod liver oil, there resulted a thick and practically normal enamel and dentine.

Mrs. Mellanby has pointed out that these cereals which interfere with the calcification of teeth, when exposed to ultraviolet light, actually improve calcification. This is, of course, the result of the induced vitamin D and is the basis of that principle almost simultaneously announced by Hess of New York and Steenbock of the University of Wisconsin, that the irradiation of foodstuffs, including the cereals, brings about in them the formation of vitamin D. The effect of irradiated oatmeal, as well as of other irradiated cereals, on the skeletal tissues—of which the teeth are an integral part—has now been shown many times by Steenbock et al. (1929). I need refer but briefly to the effect of such irradiated cereals in the treatment of ricketic conditions and the value of vitamin D in aiding the deposition of calcium phosphate in such cases. As a corollary to this, we have the prophylactic use of cod liver oil and viosterol during infancy and childhood. In a practical way, Mrs. Mellanby and C. Lee Pattison (1928) have used irradiated ergosterol—that is, vitamin D—to prevent the spread of and to arrest caries in children. They claim noteworthy results, these corroborating similar findings obtained with cod liver oil, egg yolk, and extra milk.

As to the control of and the arrest of dental caries, Boyd, Drain, and Nelson (1929) report findings that are sufficiently suggestive to quote a few lines from their final paper:

"In correlating the diets of these four groups of children, it is apparent that they differed widely, yet seem equally efficient in arresting caries. In one, calories were supplied principally as simple sugars, and fats were avoided as much as possible; in

another, fats furnished most of the energy, and only enough carbohydrate was used to make the diets antiketogenic. In certain essential features, however, the diets were similar. Each child received cod liver oil, orange or tomato juice, milk, vegetables, and fruits daily, in amounts designed to meet the requirements for vitamins and minerals. The protein allowance was at least one gram per pound of body weight. Whatever the factors essential for dental integrity, they apparently are supplied by these foodstuffs."

Mrs. Mellanby has stressed the value of vitamin D, while Boyd, Drain, and Nelson (1929), on the other hand, stress no particular item or items, but it is apparent that vitamin D was supplied through the use of cod liver oil, together with vitamin A, while vitamin C was in the diet through the use of orange or tomato juice.

Vitamin C may be called a "circulatory stimulant." Hanke (1930) has pointed out the marked betterment in the conditions of the capillaries in the gums through its use. This must necessarily lead to a better nutritional environment of any subnormal tissue.

Quite recently, Dr. Hanke reported at the annual meeting of the National Dental Association at Memphis the results of a two-year feeding test which he carried on at Mooseheart, Illinois. This is one of the most outstanding pieces of large scale dietary control work ever done and its objective was to study especially the effect of orange and lemon juice in dental caries. We all know that in such work the larger the number of subjects the better, but because of the tremendous expense involved, it falls to the lot of but few workers in this field to carry on the research on such a large scale. During the one year in which oranges and lemons were used as a source of vitamin C, 6 oranges and 1 lemon were fed daily to each of the 352 children under observation. This meant the use of over 128,000 lemons and over 750,000 oranges.

At the outset of the first year, the mouth of each child was put in perfect condition by doing the necessary dental work; X-rays were taken and also blood calcium and phosphorus determinations made. These children were kept on the regular Mooseheart diet for one year and the average daily intake for each child was 1 quart of milk, 9/10 lb. of vegetables, 1/2 lb. of fruit other than citrus, 6/10 of an egg, 1 oz. of butter and 1/4 lb. of meat. These daily averages were calculated from the whole year's intake. This diet is inadequate in vitamin C, and somewhat so in both D and A. At the end of the first year, all but 88 of these children had developed caries and 223 of them had some form of gingivitis.

During the second year of the experiment, the diet was exactly the same as the first year, except that there was added to it the daily intake of one pint of orange juice and the juice of one lemon. At the end of the second year, 95% of the gingivitis had disappeared and caries had been arrested in 60% of the cases of those who had had

caries develop the first year. This shows quite clearly the effect of orange and lemon juice in the arrest of caries. We can only conjecture what the effect of an adequate vitamin D and A intake might have been in the control of a part or all of the remaining 40%. While orange and lemon juice are rich in vitamin C, it is only through the feeding of a *pure* vitamin C that it can be determined definitely whether or not these results are due solely to the C content of these juices, or whether some other factor is involved.

For experimental work on the antiscorbutic vitamin—vitamin C—the guinea pig is usually employed, for it is, like man, quite susceptible to diets where it is absent or only present in inadequate amounts. The dental integrity of the teeth of guinea pigs, so far as this particular vitamin is concerned, may be maintained on 3 cc. of orange juice or 1 cc. of lemon juice daily. When deprived of this vitamin there is a derangement of the odontoblastic layer. If the scorbutic diet is fed for a few weeks, the odontoblasts may disappear entirely, the pulp chamber become hemorrhagic and the supporting bony tissues show osteoporosis. So delicate is this test, in the case of the guinea pig, that a difference in tooth structure may be seen between a daily intake of 3 cc. of orange juice and that of only 2.5 cc. (Eddy, 1931).

From what has been said, we must not get the idea that vitamins C and D tell us the whole story. Adequate amounts of vitamin A have been shown by Howe (1929) to play a part in the formation of sound teeth. An insufficient amount of this vitamin in the diet causes the odontoblasts to lay down bone rather than normal dentine. Recent observations seem to point to the fact that vitamin B also is essential. Neither should an adequate mineral intake be overlooked. So it may be seen that this problem is not so simple as it might be, but the various nutritional factors for building sound and resistant teeth are being studied and eventually we will get a still more complete and satisfactory picture.

While it may seem that an undue portion of this paper has been confined to dental problems, it has been done with the thought of bringing to your attention one of a nutritional character in which the effects of a deficient diet are strikingly obvious. Yet it is equally true, even though less obvious, that such deficient diets may be reflected elsewhere in subnormal conditions.

There are six to seven pounds of inorganic or ash-forming constituents in a 150-pound man. This is made up largely of calcium and phosphorus, for the body contains about 2% of calcium and 1% of phosphorus. These percentages of calcium and phosphorus are emphasized here, for it gives us a ratio of 2 to 1 for these elements in the body.

In the bodies of all animals, man included, are a large number of other elements, which are quantitatively insignificant as compared with the calcium and phosphorus. This does not mean, however, that they are physiologically insignificant. In fact, we know that some of them are significant. These little cogs in the complex mechanism we call health can no longer be ignored. If these little cogs cease to function perfectly, the mechanism as a whole may still operate, but the output in terms of health is at a lower level and the result is anything from the feeling that one is not quite "up to the mark" to a distinct pathological lesion.

What a beautiful and significant piece of work was that of Hart (1928) and his associates (1929), at the University of Wisconsin, when they found that mere traces of copper were needed for iron to exercise its hematopoietic function—its power to build hemoglobin. What a wonderful nutritional balance, if so it may be called, there is in Nature under normal conditions. This may be exemplified by the fact that the new born carry three times as much iron as they do in the adult stage—at least that has been shown to be true with the newly born of the human species, with puppies, kittens, and rabbits. Calves also carry a relatively large concentration of copper, as compared with the adult, and this mobilization of copper in the embryonic stage is undoubtedly of biological significance (McHargue, 1925). This mobilization of iron, however, is to tide the young over the suckling period, for as McHargue (1925) has pointed out milk is notoriously poor in this element. There is, however, a remarkable similarity of the mineral constituents of the young and of the milk of the mother. This concentration of iron is not to be found in the young of guinea pigs, for they are born with teeth and feed like adults practically as soon as born. What can we say about the fact that the new born also carry three times as much zinc as is found in later life? About the significance of this we can, at present, say practically nothing, for whatever the function of zinc may be, little is at present known about it.

According to the views of Dr. Orr (1925) of the Rowett Research Institute:

> "It is probable that the functions of the bones, viz., regulating the supply of mineral elements to the body fluids, is as important as the more obvious one of providing a rigid framework. It is probably more fundamental, for, when the available mineral matter is insufficient to maintain the physiological balance in the blood and the rigidity of the skeleton, it is the rigidity which is sacrificed. There is little doubt that in most diseases affecting the bones, the skeletal symptoms are only secondary manifestations of the influence of some factor, often a dietary one, which upsets the balance of the mineral elements in the blood."

It has been estimated that a cow may lose as much as 20% of the

mineral matter of the skeleton during the lactation period. Orr has suggested that—

> "this depletion of the tissues of heavily milking cows may be the cause not only of decreased milk yield in subsequent lactations, but of difficulties in breeding and an increased susceptibility to disease."

During pregnancy, the expectant mother has trouble with her teeth, which are a part of the skeletal tissue, for her mineral intake and retention are inadequate to meet her needs. Cows which are heavy milkers are usually in negative balance—their mineral intake being less than the mineral outgo—and this difference is supplied by the mineral reserve, the skeleton. When our mineral intake and outgo do not at least balance, we are drawing on our reserves, and if this is long continued, our reserves become depleted and that spells disaster of some sort.

It is, I think, justifiable to look at the character of the mineral ingredients found in the bones, to learn something of what the body may demand in time of stress; it is the natural thing for just those elements to be laid down in this tissue that the body may need and, moreover, laid down in just the proportions needed. In the first place, the calcium to phosphorus ratio in the skeleton is about 2 to 1—about the same as in the total body ash, to which ratio attention has already been directed. In the bone, besides calcium and phosphorus, are found such elements as iron, copper, zinc, manganese, silicon, arsenic, fluorine, and magnesium. So far as I am aware, no one has ever made a careful and complete spectrographic study of bone. Dr. Howe (1926), of the Forsyth Dental Infirmary, did do this in a limited way with teeth, and he reported the presence of boron. He said that such a finding was an indication that the person from whom the teeth came had been using a boric acid mouth wash. While this may have been true that boric acid had been used, it is also a fact that boron is widely distributed in the animal body. When a careful spectrographic examination of bone is made, there will be disclosed small amounts of elements, other than those which have so far been found.

The inorganic elements have a fundamental role to play in nutrition. So far as the skeletal tissue is concerned, the first line of defense in our mineral reserves lies in the bone trabeculae. This has been shown in a splendid fashion by Bauer, Aub, and Albright (1929) of the Massachusetts General Hospital.

These experimenters placed some cats on a high calcium diet and some on a low calcium diet. The high calcium diet consisted of milk and, in one animal, of milk and raw eggs. The low calcium diet consisted of cooked meat, fresh raw liver and water; cats stay in excellent condition on this diet, even if it is given them for many months. At

the end of several months, the left fore leg was amputated at the shoulder joint under ether anesthesia; then the diets were reversed. After a similar period of time, the animals were sacrificed, thus allowing comparison of the humerus representing the high calcium diet with the humerus of the low calcium diet period. This comparison showed conclusively that the trabeculae can be built up in passing from the low to the high calcium diet; conversely, they disappeared when these diets were reversed. The disappearance of the trabeculae or their formation is a function of the time element involved, as shown in the case where the low calcium diet was maintained for 379 days. Here the trabeculae had not only almost completely disappeared but there was also a disappearance of the majority of the epiphyseal end of the bone.

This building up, as well as disappearance, of the trabeculae was also beautifully shown by the daily administration, either orally or intramuscularly, of Alizarine Red, which stains newly forming trabeculae. If, after the use of the dye, it is withdrawn, and high calcium feeding continued, white trabeculae are formed, representing the trabeculae laid down during the period that the animal received no dye.

At this point, mention should be made of some work published last year by Salter and Aub (1931). They found that—

> " calcium fails to be deposited in bone when the diet, though adequate in calcium, is deficient in vitamin C. The subsequent addition of C to such a diet allows calcium to be rapidly deposited. This deposit is largely at the epiphyseal ends of the bone and in the trabeculae. In other words, not only growth, but also the stores of the reserve supply of calcium are involved by pathologic changes in the bone cells."

During the countless thousands of years of evolutionary development through which man and other animals have passed, there must have been an adjustment—an establishment of an equilibrium—between man and the elements which constituted his food. Man had to adjust himself to his environment and he developed accordingly. There is really quite a remarkable similarity between the mineral requirements of man and of plant life. They are not exactly alike, of course, neither is such to be expected. Man's requirements for minerals are met, however, either directly or indirectly from plant sources.

We now know from McHargue (1922, 1926, 1927) that manganese is essential for the development of the chlorophyl in plants. The fact that manganese is necessary in plant growth has been pointed out by Schreiner and Dawson (1927). That it is found in largest concentration in plant and animal tissues which contain the greatest vitamin potency, has led McHargue to assume that a relationship exists between this element and the vital factors contained in these tissues.

The largest concentration of manganese in animal tissues is found in the liver and next in the kidneys.

It may not be without significance that, compared with the vital organs, manganese occurs in still greater concentration in commercial preparations of digestive enzymes, pepsin, rennin and trypsin.

The vital necessity for manganese in animal life is now becoming clearer since the recent work of Orent and McCollum (1931) of Johns Hopkins University. They found that on a manganese-free diet the female rats would conceive and bear young in a normal way, but after the birth of the young, the mother lost all maternal instinct and the young died of neglect. On a manganese-free diet, the male sex glands degenerate until complete sterility results. Addition of as little as five-thousandths of one per cent of manganese results in correcting the behavior of the female rats towards their young. Orent and McCollum suggest that dietary manganese may in some way be related to hormone formation of the pituitary gland.

McCollum and Orent (1932) have also determined that magnesium is essential to a normal diet, at least in the case of rats. In less than two weeks, on a magnesium-free diet, the experimental rats went into spasms and most of them died. According to McCollum and Orent, magnesium is necessary in the diet, being essential to the proper functioning of the adrenal glands. The need of magnesium for normal plant growth is well known.

The soil of the famous Kentucky blue grass region is the richest soil in that State, not only in copper and manganese, but also in zinc, nickel and cobalt. The presence of these elements in the soil has been shown by McHargue (1927) to be reflected in the mineral constituents of the blue grass grown on it. The luxurious growth of this grass is famous and is reflected in the development of live stock in this region, for which it has attained a world-wide fame.

The mention of cobalt and nickel in the ash of the Kentucky blue grass brings to mind Bertrand's views of a concentration of these elements in the pancreas of animals. He claims that these metals have an effect similar to that of insulin in the metabolism of sugar in diabetes.

The investigations of Schreiner (1929) in Florida furnish a striking example of the great value in plant growth of minute quantities of the rarer elements, especially copper. Allison et al. (1927), in working with Everglade peat, obtained growth response on a long list of plants, using such unusual fertilizing elements as zinc, antimony, nickel, tin, barium, copper and manganese. Of these, the most favorable results were obtained with copper and the residual effects have carried over for more than a single cropping season. Contrasted with this finding

of the value of copper in plant growth is its value in human nutrition, which has already been mentioned.

With respect to boron, we know it is essential, too, in minute amounts for the development of plants. In legumes, for example, Schreiner (1929) has shown that it exercises an important function in connection with the nitrogen fixation process. The value of boron in human nutrition is still a riddle.

Before leaving this most fragmentary discussion of minerals, mention should be made of their presence in wheat. One of the members of our Association of Cereal Chemists has been quite actively engaged in the study of this problem for some years. Through the courtesy of Miss Betty Sullivan, I am able to refer to some of her, as yet, unpublished findings in this field. For several years she has been studying this problem from a spectrographic standpoint and has identified, in wheat, the presence of such elements as zinc, manganese, cobalt, arsenic, iron and boron, as well as many other elements such as silver, copper, chromium and aluminium. This method of attacking the problem by spectrography is fundamental, not only from the purely cereal point of view, but from the nutritional as well. As to the latter, its value is, to a degree, potential, for the role which some of these elements play in nutrition is for the future to determine.

If you are interested in this matter of copper, manganese and iron, in relation to their amounts in serving portions of food, you will find this splendidly worked out in an article by Hodges and Peterson (1931). From the tables given, it may be seen that cereals contribute the largest proportion of manganese and copper to the diet and that vegetables come next. Cereal and vegetables supply about the same proportion of iron.

I have taken occasion to refer twice to the subject of a calcium to phosphorus ratio of two to one. I believe it to be accepted by nutrition experts that the calcium should exceed the phosphorus in the diet, yet the numerous studies made of American dietaries cited by McCollum and Simmonds (1929) shows that this is not the case. In wheat flour, the phosphorus calculated as P_2O_5 makes up approximately one-half the ash, while the MgO varies from 10% to 17% and the CaO ranges from 2.5% to 4.5%. In other words, if the calcium to phosphorus ratio is calculated from these figures, the phosphorus is found to far outweigh the calcium. In fact, practically the whole list of cereal products shows this unfavorable ratio of calcium to phosphorus. If calcium is taken as unity, the phosphorus values in some of these are as follows (Schneider, 1930):

TABLE I

RATIO CALCIUM TO PHOSPHORUS. CALCIUM = 1

White wheat bread	3.44	Shredded wheat	7.90
Whole wheat bread	3.50	Barley grits	9.05
White wheat flour	4.60	Corn meal	10.55
Oatmeal	5.68	Polished rice	10.67
Farina	5.95	Hominy	13.09
Rye bread	6.16	Wheat germ	14.79
Macaroni	6.55	Rye flour	16.06
Whole wheat flour	7.67	Sweet corn	17.16

While these are in themselves unfavorable ratios, that does not mean anything else than that these ratios can and must be modified in our diet by the ingestion of calcium-rich foods. These unbalanced ratios found in cereals call to mind conditions found in foods with respect to their proteins. No single foodstuff is complete with protein of such a character that it yields all the necessary kinds of amino-acids for carrying on life processes, hence the need for a mixed diet.

You are all aware of the great value of vitamin D, ultraviolet light and sunshine in promoting bone growth in rickets, also in respect to health in general. Vitamin D does have the power of vitiating the bad effects of unfavorable calcium to phosphorus ratios in our food. And it may be said further that the nearer the correct ratio of calcium to phosphorus, the less the need for vitamin D. What this vitamin seems to do is to raise the cellular activity in some way—to raise its potential, so to speak—so that the retention of minerals, especially of calcium and phosphorus, more nearly approaches the normal, notwithstanding that the diet may be abnormal in that respect, or inadequate in amount. We must not, however, by our overconfidence in the value of this antirachitic factor—vitamin D—neglect to supply the adolescent with an ample and liberal supply of calcium and phosphorus and to supply them in a favorable ratio. This is equally true after adolescence is past. I fear these two principles are too much neglected when cod liver oil or viosterol is given. Sherman (1930) has put it in the following fashion:

" Recent enthusiasm over vitamin D as a ' calcium conserving ' factor has led some to take a less careful attitude towards the problem of calcium supply; but no relaxation of attention to calcium is justified, for a careful study of the evidence shows plainly that vitamin D cannot take the place of calcium in nutrition; it should be regarded as a ' calcium mobilizing ' rather than a ' calcium conserving ' factor. It prevents and cures rickets by restoring the calcium content to the blood where this has been subnormal and by favoring the deposition of calcium phosphate in the ends of the bones; obviously this is not a creation but rather a diversion of calcium—sometimes a diversion from the shaft of the very same bone! Under vitamin D treatment, therefore, rickets may be prevented or cured while the body as a whole remains calcium-poor."

As Corlette (1928) has put it:

" The ' demand ratio ' for calcium and phosphorus is broadly parallel to the ratio in which these elements are stored in the body."

Experimental work, looking to the isolation not only of vitamin D but of the other vitamins as well, is being intensively carried on in this and other countries, such isolation being necessary before work on chemical identification can be undertaken. Claims for the isolation of vitamin D in crystalline form have been made by Windaus (1931) and his associates (1931) in Germany, and by Bourdillon and his associates (1931) at the National Institute for Medical Research in London. The latter product has been given the name of "Calciferol." The experimenters to whom reference has just been made started with irradiated ergosterol as the source of the vitamin D. In December, 1931, at the annual meeting of the American Association for the Advancement of Science, Bills and McDonald described the preparation of a very potent crystalline vitamin D, in the preparation of which irradiation played no part, the method used being wholly of a chemical character and dependent on the action of nitric oxide on solutions of ergosterol with the rigid exclusion of oxygen. Late in 1931 came the announcement by Rygh, of the University of Upsala, that he had succeeded in obtaining vitamin C in crystalline form. So far as vitamin A is concerned, Drummond, Heilbron and Morton have just announced the splitting of carotin into two parts, one of which is believed to be vitamin A, or at least its precursor. This split portion is an alcohol, contains no nitrogen and has a potency about that of the newly discovered crystals of vitamin D. With regard to carotin—the coloring matter of wheat flour—it is interesting to note that the Committee appointed by the League of Nations (Editorial) to formulate standards to be used for the biological assay of the various vitamins has adopted a specially purified carotin as a standard for assaying products for their vitamin A potency.

The blood calcium from birth to "three score years and ten" is almost a constant quantity. There is a slight decrease, but not a large one according to Greisheimer et al. (1929). With the inorganic phosphorus it is a different story. At birth, the inorganic phosphorus in the blood is about 5.6 milligrams per cent, that is, 100 cc. of the blood serum contains about 5.6 milligrams of inorganic phosphorus. At about twenty years of age—say at the end of the adolescent or growth period—this figure of 5.6 has dropped to about 2.75 milligrams per cent. It remains practically constant from this point through the remainder of life. Such figures as have been given must not be considered as absolute. Eddy and Heft (1923), for example, give the latter figure as 2.9. What is to be remembered is that there is a very significant drop in the inorganic phosphorus at the end of the growth period.

When a fracture occurs in the case of an adult, if there is normal

bone repair of such a fracture, the inorganic phosphorus increases for the time being to about that found in the blood during early childhood. In other words, the calcium and phosphorus are used in making bone for the repair of the fracture, just as they are used for making bone for growth and are present in the blood in about the same concentration. If, however, the inorganic phosphorus fails to increase, then we have a non-healing fracture.

Peterson (1924) points out that if the product of the calcium times the phosphorus is less than 30, fractures will not unite. If the product in the case of children is below 30, rickets is invariably present. When the healing occurs as the result of any therapeutic measure, the product will be found progressively to rise (Howland and Kramer (1922)). This betterment in the condition of the blood inorganic phosphorus can be brought about by proper diet. Even if mineral reserves are readily available in the body, the physiological preference for phosphorus retention is from the food ingested.

The need for vitamin D does not seem to be as clear cut in the case of adults as in the case of the young. But in certain abnormal conditions in adults, its value has been shown—as, for example, in osteomalacia, which is in the same category as rickets in children.

The changes in the inorganic blood phosphorus are shown graphically in Figure 1. This figure, as well as Figure 2, has been taken from

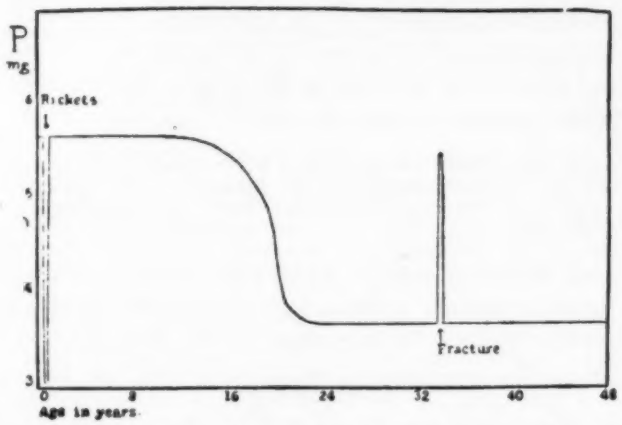

Fig. 1. Changes in the inorganic blood phosphorus level from birth, through the adolescent period and into later life.

the work of Tisdall and Harris (1922). In Figure 1 are shown the changes in the inorganic blood phosphorus level from birth, through the adolescent period and into later life. The peaking of the phosphorus during the normal repair of a fracture is also shown, as well as the reverse of this—its decrease in rickets, a disease of very early childhood.

In Figure 2 is shown an example of the actual changes in the inorganic blood phosphorus during the repair of a fracture and its return to the normal level after the repair work has been accomplished. It

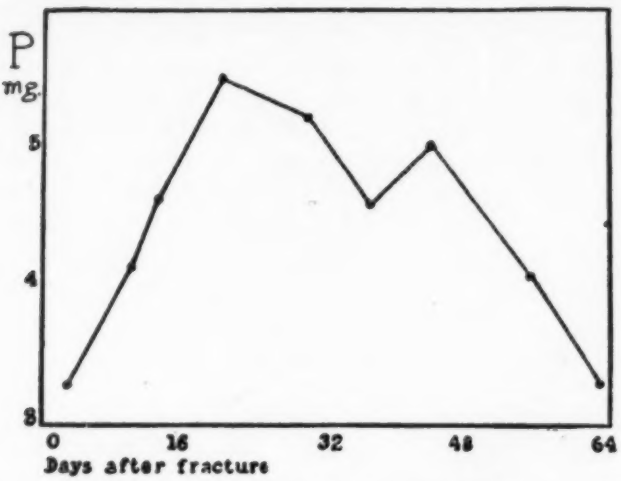

Fig. 2. Changes in the inorganic blood phosphorus during the repair of a fracture and its return to the normal level after the repair work has been accomplished.

is not without significance that this peaking of the inorganic blood phosphorus in adults also takes place during the recovery from an operation on the soft tissues.

Quite naturally, we all wish to live as long as we can and to be as mentally and physically vigorous as possible. As to length of life to quote from Sherman and Campbell (1930):

"... it may be regarded as established beyond any reasonable doubts that, starting with a diet which is already clearly adequate, it may still be possible to induce a very significant improvement in longevity by enriching the diet with certain of its chemical factors."

It is worth our while for each of us to give, from time to time, some attention to such matters as have been considered in this paper, for after all is said and done, we are largely what we eat.

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THE HYDROLYSIS OF STARCH IN BREAD BY FLOUR AND MALT AMYLASE

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(Read at the Convention, May, 1931)

Introduction

It has long been known that the staling of bread is closely associated with the degree of hydration of the starch. The first quantitative study was probably made by Lindet (1902), who determined the variation of soluble amylose during storage. He also introduced a criterion named the "coefficient of absorption"; essentially the ratio of the density of crude starch to that of the bread starch. Katz (1912) developed three methods for the study of this phenomenon, (*a*) a "hardness" test, (*b*) determination of the amount of soluble starch, and (*c*) the "swelling power" of residual starch. The "hardness" test, being the most rapid and convenient of the three, was further developed by Platt (1930), and Bailey (1930) into a comparatively exact method. Staleness is measured by noting the penetration of a weight or plunger into the crumb, i.e., "compressibility." Peper (1926) describes a variation of Katz's "swelling power" test. Karacsonyi (1929) has developed a method involving the viscosity of 10% suspensions of bread crumb which is stated to be convenient and capable of considerable accuracy. Katz (1931) has also recently applied the methods of X-ray spectroscopy to the problem.

Experimental Procedure

Methods. In view of our scanty knowledge of the fundamental nature of staling, any new method which promises to throw light on the mechanism may be considered welcome. That susceptibility to diastatic attack is related to the degree of gelatinization of the starch is well known. The convenient method for the study of diastatic action as developed by Schultz and Landis (1932) was applied to the problem. This in brief was as follows:

A quantity of starch bearing material, buffer and yeast are placed in a reaction bottle and the solution saturated with carbon dioxide by the addition of a small amount of maltose. A diastatic enzyme is then added; the yeast ferments the sugar as rapidly as it is formed. The gases evolved during the fermentation are collected and measured at convenient intervals. The solution is continually shaken to prevent supersaturation by CO_2. The amount of gas formed bears a stoichiometrical relationship to the sugar or starch in the reaction bottle.

Materials. Non-diastatic and diastatic loaves were baked by the following formulas:

	Non-diastatic	Diastatic
Hard wheat flour	300 gms.	300 gms.
Water (about)	200 gms.	200 gms.
Yeast	3 gms.	3 gms.
Diastatic malt extract	0	3 gms.
Salt, sugar	none	none

After baking the loaves were cooled in air (except the 4 and 8 hour loaves, which were placed almost immediately in the ice box), and two hours later were wrapped and stored at various temperatures. At intervals the loaves were cut open, 20 gm. portions of the crumb were placed in reaction bottles, and 90 cc. water, 5 gms. yeast and 10 cc. buffer [1] added. After the sugar in the sample was fermented 0.5 gm. flour was added. The volume of gas evolved when the rate of evolution became practically zero was taken as a measure of the amount of starch in the crumb attackable by the enzyme. At this point a small amount of malt extract with extremely large liquefying and saccharifying power was added, whereupon an additional amount of gas was produced. The total amount of gas thus obtained was approximately the same for all loaves; viz., 1200 to 1250 cc., and apparently represents the limiting amount of gelatinized starch in the loaf susceptible to attack. Complete gelatinization of the starch by boiling with excess water before enzymic decomposition did not change this figure. Upon complete acid hydrolysis of a 20 gm. portion of bread, previous to fermentation, 1900 cc. of gas were formed. Hence the usual 60 to 65% of the total polysaccharides present at any stage of staling are attackable by the malt diastase, but not by the flour diastase, an anomaly in which cytolytic activity probably plays a part.

Discussion

The amount of starch attackable by the flour diastase as measured by the gas evolved is given for a number of temperatures and storage

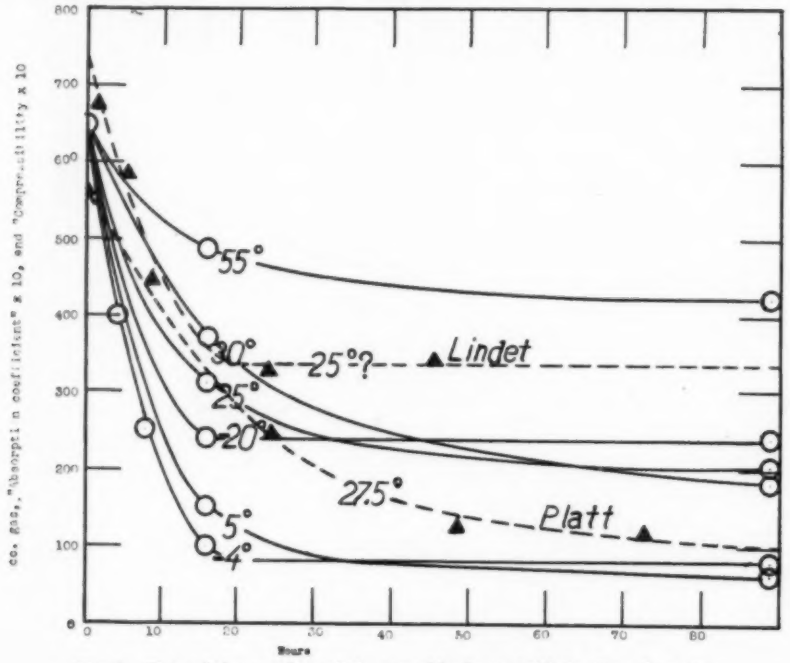

Fig. 1. Rate of change of starch in normal loaf attackable by flour diastase.

[1] 100 gms. potassium citrate, 20 gms. citric acid, and 20 gms. ammonium acid phosphate per liter of solution. pH 4.5 to 5.5.

times in Figure 1. If these data be taken as a measure of staleness, it is obvious that the staling proceeds at a very rapid rate for the first 10 to 15 hours. Apparently, at this time, equilibrium or suspended action is nearly reached, particularly in the case of the lower temperatures, for but slight change occurs thereafter. It is remarkable that the "compressibility" curve at 27.5° C. plotted from the data of Platt (1930) parallels fairly closely the gas curve at 30° C.

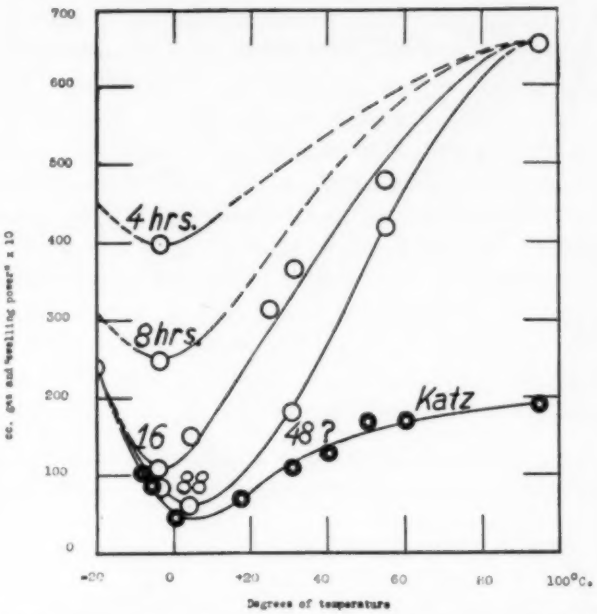

Fig. 2. Variation in temperature of storage with amount of starch attackable by flour diastase.

These results are shown again in Figure 2, the gas evolved being plotted as a function of the storage temperature. A definite minimum is apparent near 0° C. Attention may be called to the fact that the swelling power, as determined by Katz (1912), follows the same general trend. The existence of this minimum is probably due only to the fact that the rate of change below this temperature is very slow and that true equilibrium does not prevail. For example, if the loaf could be cooled instantaneously from oven temperature to −20° C., theoretically no staling should be apparent, the system having no time to approach equilibrium.

These data for the diastatic loaf are shown in Figure 3. While the rate of staling is approximately the same, the extent of the change is less, particularly for the lower temperatures. Thus, at 5° C. nearly twice as much attackable carbohydrate appears to be present. The

crumb texture also indicated that staling had not progressed to the same extent as in the non-diastatic loaf. The curves for 55° C., however, are nearly identical. Diastatic loaves stored at −4° C.

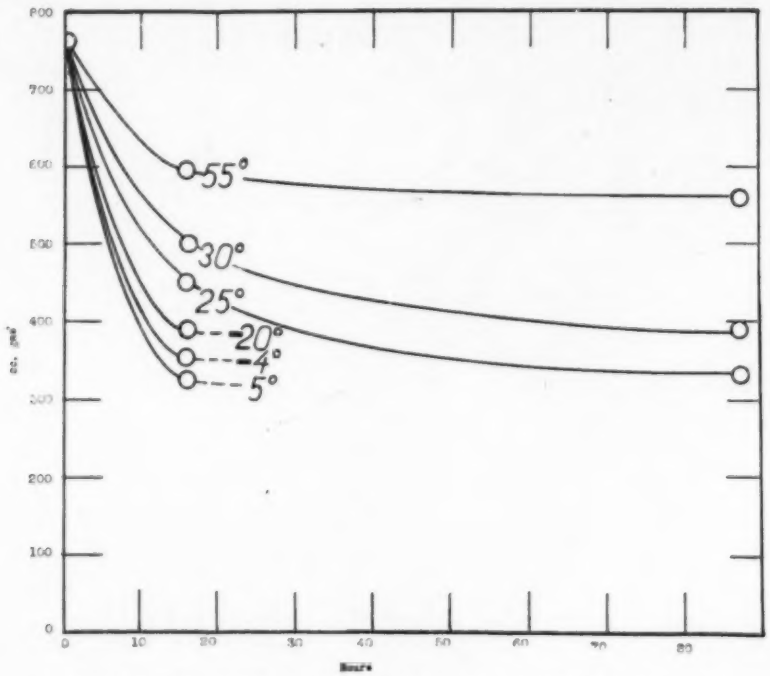

Fig. 3. Rate of change of starch in diastatic loaf attackable by flour diastase.

for 88 hours (giving 370 cc. gas) were subjected to temperatures of 55° C. for 24 hours and 100° C. (oven) for 0.5 hour and immediately tested. The volumes of gas were 515 and 770 cc. respectively, approaching those obtained from the loaf stored at 55° C. and the fresh loaf. Hence, the same equilibrium point is reached from low as well as high temperatures, substantiating the reversibility of the process.

On the Theory of the Role of Starch in Staling

As Alsberg (1928) has noted, the "retrogradation" of starch as applied to reversion of starch paste or autoclaved starch solutions seems to proceed much more slowly than the staling of bread, and, in addition, the process is not reversible. Katz (1912) has argued from the fact that the equilibrium position depends upon the temperature that a physico-chemical equilibrium is involved. Taylor and Beckmann (1929) have shown that the viscosity of a starch water mixture varies in a regular manner with the temperature of the mixture and the swelling and degree of disintegration of the granules. The maximum

viscosity is attained during, or immediately after, the disintegration. Under conditions existing in the interior of the dough in the oven, it is highly improbable that any of the granules actually reach the bursting point, although they are considerably swollen. In this state the matrix would be relatively viscous and the crumb tenacious and somewhat sticky in accordance with the observed characteristics. Since this swelling or imbibition of water is *reversible* and has been shown to be a function of the temperature, it is obvious that upon cooling a decrease in the size and water content of the starch granules and a consequent decrease in the viscosity of the matrix and tenacity of the crumb occur.[2] Air spaces have been observed between the shrunken granules and the gluten matrix (Verschaffelt and van Teutem, 1915). If, while in the swollen condition, the starch granules are subjected to extraction with a large amount of water, a certain amount of amylose will go into solution, but this amount will decrease in a regular manner with a decrease in the degree of swelling. Moreover, dry or but slightly swollen (partially retrograded) starch is attacked by flour diastase, only with considerable difficulty. These concepts are roughly illustrated in Figure 4. It is believed that this rather simple explanation will coincide with most of the observed facts concerning the difference in the properties of fresh and stale bread.

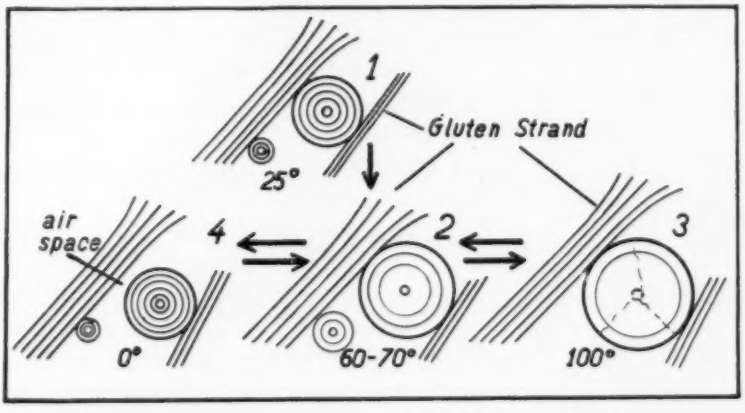

Fig. 4. Diagrammatic picture of the structure of starch in bread. (1) Dry granule in the dough; (2) Partially swollen granule existing during oven spring; (3) Swollen granule at end of baking period; may reach incipient rupture; crumb elastic and sticky; (4) Shrunken granule in stale bread; crumb stiff and harsh.

Summary

The amount of starch in normal bread, baked without diastatic malt, attackable by *flour diastase* is shown to decrease very rapidly

[2] Katz (1931) has recently applied X-ray spectroscopy to this problem, and has shown that a characteristic *reversible* change in lattice structure accompanies staling. The term "retrogradation" is applied to this phenomenon, and in this sense may be used to describe the change occurring.

during the first 10 to 15 hours of storage, and to reach a minimum in the neighborhood of 0° C. The results obtained by this method of observing the change appear to coincide closely with those obtained by other methods. The amount of starch in bread attackable by *malt diastase*, however, is found to be much greater and practically independent of the manner of storage. The addition of small amounts of diastatic malt extract to the dough is shown to increase the amount of attackable carbohydrates over those of the normal loaf, particularly at lower temperatures of storage.[3] The theory of the role of starch in bread staling as associated with reversible swelling of the starch granule, probably accompanied by structural changes, is elaborated.

Acknowledgment

We wish to acknowledge the cooperation and assistance of Dr. Charles N. Frey, Director of the Fleischmann Laboratories, in the study of this problem.

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[3] Bailey (1932) has substantiated the retarding effect on staling of malt extracts, using the "Compressibility" method.

VARIATION IN THE WEIGHT OF A GIVEN VOLUME OF DIFFERENT FLOURS [1]—I. NORMAL VARIATIONS

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(Received for publication March 16, 1932) [2]

Introduction

The common practice of measuring ingredients by volume and not by weight leads to numerous difficulties in baking. Some of these baking difficulties are due to faulty technique in taking volume measurements, and others are due to lack of correlation between the volume taken and the weight of different samples of the same material. That there is a marked difference in weight between hard-wheat and soft-wheat flours is generally recognized by flour manufacturers and bakers, and in experimental laboratory work. One cupful of soft-wheat flour, for example, weighs considerably less than the same volume of hard-wheat flour. In most experiments with batters and doughs, the weights used for the two flours are those selected by Woodruff; [3]—100 gms. per cup for soft-wheat flours and 113 gms. per cup for hard-wheat flours.

In addition to the fact that a given flour may vary greatly in weight per unit of volume taken, depending on the method of handling and taking the volume, an important consideration that does not seem to be generally recognized is that the hard-wheat flours and soft-wheat flours may differ considerably among themselves in the weight of a given volume.

Preliminary experiments with some pastry flours, conducted at the University of Chicago, showed that the difference in quantity of flour taken when the flours were placed in a given volume and then weighed, might be as great as two tablespoonfuls per cup. As this variation is large enough to account for some of the failures in baking made in changing from one brand of flour to another, further studies were undertaken in order to determine how wide a difference is to be found in the weights of a given volume of different types of flour and also with respect to different flours of the same type. Tests were also made to determine the influence of such factors as the settling of flour

[1] This investigation was started when the author was a student at the University of Chicago, and completed in the Bureau of Dairy Industry, U. S. Dept. of Agriculture.

[2] Read at the Convention, May, 1930.

[3] S. Woodruff, 1922. Weights of one cupful of food materials. J. Home Econ. 14: 270–274.

before use, and the effect of flour packing in the container after it had been sifted for use, upon volume weight.

Experimental

Method Used in Experiments. Two pounds of flour were sifted into a bowl. Nine heaping tablespoonfuls of flour were resifted into a second bowl, care being taken not to jar the bowl while sifting. This twice-sifted flour was dipped up in heaping tablespoonfuls and gently placed in a cup until the cup was full. Then the flour was levelled off with one sweep of a spatula held at right angles to the top of the cup, and the cupful was weighed. This process was repeated five times, a cupful of newly sifted flour being used each time. The method of handling gave very uniform results. The average deviation of the mean, calculated on 80 flours, was found to be 0.651 gm. per cup of flour.

Results of Experiments. The average weights per cup for a large number of hard- and soft-wheat flours made from wheat grown in the period 1926–1929, representing the four leading wheat-producing regions of the United States, are shown in Table I. Samples of wheat were obtained through the courtesy of many millers in different sections of the United States. In all, 323 samples of commercially milled flour were tested and the results included in preparing the group averages given in this table.

The large mills usually obtain their supply of wheat from a distance, but many of the smaller ones obtain most of their supply from comparatively near-by localities. Most of the soft-wheat flour used in this investigation came from mills of about 500-barrel capacity. The data given, therefore, pertain to the state in which the wheat was milled and not necessarily to the state in which it was grown.

TABLE I

AVERAGE WEIGHTS OF A CUP OF FLOUR FROM THE FOUR LEADING WHEAT-PRODUCING REGIONS OF THE UNITED STATES; AND SAMPLES TAKEN FROM 1926–1929 CROPS

State in which wheat was milled [1]	Kind of wheat [2]	Weight per cup of flour			
		1926	1927	1928	1929
		gms.	gms.	gms.	gms.
Missouri, Illinois, Indiana, Ohio	(a)	93	92	93	93
Michigan	(b)		86		90
Pennsylvania	(a)			87	
Maryland	(a)			83	
Washington, Oregon, Utah	(b) & (c)	85	89	90	90
North Dakota	(c)	111		114	110
Kansas	(d)	114		112	111
Oklahoma	(d)			111	

[1] Because of the shortage of soft red winter wheat in Missouri, Illinois, Indiana, and Ohio in 1927 and 1928, the four states are grouped together.

[2] (a) Soft red winter, (b) white, (c) hard red spring, (d) hard red winter.

Very little variation from year to year was found in the average weight of flour made from wheat milled in the same locality. The average weights of all the hard-wheat flours tested, which were from mills in North Dakota, Kansas, and Oklahoma, were approximately the same, ranging from 111 to 114 gms. per cup.

Flour from soft red winter wheat milled in the eastern part of the East Central section was lighter in weight than that milled in the western part. Flour from white wheat milled in Michigan weighed less than that from soft red winter wheat milled in the near-by states.

Flour from wheat milled in the Pacific Northwest averaged about 88 gms. per cup, but on the basis of weight there were two distinct types of flour from that section. Many samples weighed about 82 gms. per cup, whereas others weighed about 90 gms. Causes of this difference may be that in these states some wheat is grown on irrigated soil and some on non-irrigated soil, and some flours are milled from white wheat and other flours from red wheat.

Of the soft-wheat flours, the heaviest came from Missouri and weighed 98.7 gms. per cup, and the lightest came from Maryland and weighed 79.9 gms. for the same volume, a difference of 18.8 gms. This difference is equivalent to 3 tablespoonfuls of the lightest flour and consequently has a real significance.

Frequently a variation of from 7 to 10 gms. was found in the various samples used in computing the averages given in Table I. This fact partly accounts for the difficulties the home bread maker has in changing from one brand of flour to another.

Variation in Weight of Flour When Handled by Different Individuals. In preliminary work it was observed that when different persons took a cup of the same flour and weighed it, the weights did not check very closely. On account of this variation it was believed desirable to compare the results obtained by the writer with weights of samples taken by other individuals, in determining an average weight for soft-wheat flours. In this work forty-five testers participated who were students of the Universities of Minnesota and Chicago.

The results obtained by five of the testers are shown in Table II.

Although the weights obtained by each tester agreed fairly well, considerable variations were found between the weights obtained by different testers. Since the method used was the same and the results differ, it is evident that personal element is a factor. It is very probable that these variations were due largely to the packing effect caused by heaping different quantities of flour above the rim of the cup before it was levelled off. The average weight of the cups of flour weighed by the students was 92.9 gms, whereas in the case of the writer's work the average was 93.8 gms. The same method of handling

the flour was used by both the students and the writer. This method differs from the method already described in the following respect: A 150-gram sample of flour was used over and over again for the five measurements made in obtaining the average weight per cup, instead of estimating the amount to be used and taking fresh flour for each test.

TABLE II

COMPARISON OF WEIGHTS OF CUP OF SOFT-WHEAT FLOUR WHEN HANDLED BY THE SAME PERSON AND BY DIFFERENT PERSONS

Trial number	Student No. 21	Student No. 22	Student No. 23	Student No. 24	Student No. 25
	gms.	gms.	gms.	gms.	gms.
1	97.7	92.9	92.5	98.1	93.3
2	96.4	93.6	93.5	98.0	92.6
3	97.9	91.3	93.4	95.4	92.9
4	96.7	93.2	93.5	97.4	91.9
5	97.0	92.4	92.6	94.3	95.1
Average	97.1	92.7	93.1	96.6	93.2
Greatest variation	1.7	2.3	2.0	3.8	3.2
Average variation from mean	0.51	0.65	0.44	1.44	0.90

Difference Due to Technique or Method of Handling. Experiments to determine the effect of settling before use (Table III) show that flour must be sifted at least once if fairly uniform weights per cup are to be obtained. When fresh flour is used one sifting gives fairly uniform results; but flour that has been kept for some time requires more than one sifting before it is measured out, as there is some packing and also small lumps may have formed in it.

TABLE III

EFFECT OF DIPPING AND SIFTING ON THE WEIGHT OF A CUP OF FLOUR

Trial number	Flour dipped with cup	Flour dipped into the cup with a spoon	
	Not sifted	Not sifted	Sifted once
	gms.	gms.	gms.
1	145.2	125.7	114.3
2	141.4	125.8	114.6
3	147.9	132.0	113.6
4	137.7	126.2	113.8
5	134.4	123.7	111.9
Average	141.2	126.7	113.6
Average variation from mean	4.2	2.1	0.7
Greatest variation	13.5	8.3	2.7

In order to determine the effect of flour packing in the container after it had been sifted for use, various flours were tested by sifting 250 gms. of each flour or enough to fill two cups into a container. A cup of the sifted flour from the top part of the container was 5 to 8 gms. lighter than a cup of the flour taken from the bottom part. The difference in weight between the two cups of flour depended upon the kind of flour.

In addition to variations from packing of sifted flour in the bowl or other container into which it is sifted, the quantity of flour that must be removed from the top of the cup in measuring by volume is also a factor in packing. A comparison was made of the differences in weight of the same flour when the cup was heaped approximately ¼, ½, and 1 inch above the rim. On removing this excess flour and weighing the level cupful, it was found to have had a packing effect which caused a difference of several grams in the weight of different cups of flour.

Discussion

In these experiments a variation of as much as 10 gms. per cup was found in different samples of flour from different mills in the same locality. The difference in quantity of flour per given measure of different brands is no doubt an important factor in the difficulties so frequently encountered by housewives in changing from one brand to another.

On the basis of the average weight per cup of 323 samples of commercially milled flours made from wheat grown in four different seasons, as determined by the writer, and the weights obtained when 46 different people, including the writer, took a cup of the same flour, it appears that the average weight of a cup of soft-wheat flour is 92 gms., and that of hard-wheat flour is 113 gms.

The use of weights instead of volumes in preparing batters and doughs should be encouraged in restaurants, tea rooms, etc., wherever it is practical to use weights. Weighing the proper amounts of ingredients to use in batters and doughs eliminates the element of uncertainty. If care is not used in taking of volume when weights are not taken, the ratio of flour to liquid may be greatly modified.

Two or more siftings of flour are often specified in recipes for making especially light cakes, such as angel cake. These experiments show that more than one sifting does not add to the lightness of flour, other than breaking up lumps in old flour. By taking an average of the fifth weighing and the average of the first weighing of several samples, it was observed many times, in this investigation, that repeated sifting does not increase the lightness of the flour.

From the results obtained, it would seem that the variation in weight per cup of a given flour, when handled by different persons, could partly be eliminated by sifting the flour directly into the cup. However, when flour is sifted directly into the cup, a cup filled in this way weighs less than one filled with a spoon. For this reason the method of sifting directly into the cup can not be used unless it be accepted as the standard means of filling a cup.

Summary

1. The average weights of flour made from hard winter wheat and hard spring wheat were much the same, ranging from 111 to 114 gms. per cup.

2. The average weight of soft-wheat flour milled in Missouri, Illinois, Indiana, and Ohio was 93 gms. per cup, whereas the average weight of that from mills in the Pacific Northwest, Michigan, and the eastern part of the East Central region was slightly less. This indicates that the generally used value for the weight of a cupful of soft-wheat flour—100 gms.—is too large and that 92 gms. are more nearly correct.

3. Uniform amounts of flour were obtained, only, by sifting the flour before taking the volume.

4. Packing has an appreciable effect on the weight per cup when flour sufficient for one cup or for two cups is sifted at a time. The quantity of flour that is removed from the top of the cup is also a factor.

5. Considerable variation in weight was encountered when volume determinations were made on the same flour by different individuals; however, the results obtained by each individual checked fairly well.

COMPARATIVE COOKING QUALITIES OF DOMESTIC RICES

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In a study of comparative cooking qualities of the more widely grown domestic varieties of rice, the following varieties were tested: Fortuna, Edith, Early Prolific, Blue Rose, Lady Wright, Honduras, Rexoro, and Calora. All samples were taken from the crop of 1930.

Experimental

The determinations made included the percentage of moisture in the uncooked rice, measurements of the size of the uncooked and cooked grains, the time of cooking to tenderness, the presence of hard centers, water absorbed during cooking, color of cooked rice, and an estimation of the starch in the cooking water. The determination will be repeated each year as long as the rice remains in good condition in order to observe the effects of aging on cooking quality.

The percentages of moisture of the uncooked samples were determined in the manner described by Sherman [1] for cereal products, i.e., the rice was ground to a fine granular state, heated in an oven at 98° to 102° C. until a constant weight was reached. This required heating for two hours. The percentages of moisture found in each lot are given in Table I. In this Table also will be found measurements of the size of the uncooked and cooked samples (average width and length).

The time of cooking to tenderness was taken as the time required to cook the grains until they could be crushed between the thumb and finger. The cooking was carried out by boiling the rice gently in an excess of water. Eight hundred cubic centimeters of water containing 1 teaspoon of salt were used to 80 grams of rice. The cooking containers were uniform throughout, and were used without covers. The water was first brought rapidly to boiling over a full gas flame. The unwashed rice was added gradually, and the heat reduced to maintain gentle boiling. Previous work had shown that rice is cooked best by gentle boiling. Rapid boiling breaks the grains, and cooking at temperatures below boiling permits the absorption of an excess of

[1] Sherman, Henry C. Methods of Organic Analysis, 1923, p. 334.

TABLE I

DATA FROM COOKING TESTS OF RICE

Variety	Percentage moisture, uncooked rice	Measurements of uncooked grains (average) mm.	Measurements of cooked grains (average) mm.	Time of cooking minutes	Presence of hard centers in cooked rice	Color of cooked rice	Loss of starch [1] in cooking water 1 = most
Rexoro	11.73	3 mm. wide 11 mm. long	5 mm. wide 14 mm. long	16	Not noticeable	Bluish white	2 [2]
Honduras	12.61	5 mm. wide 11 mm. long	6 mm. wide 15 mm. long	22	Not noticeable	Creamy white	1
Fortuna	11.15	3 mm. wide 9–11 mm. long	4 mm. wide 16 mm. long	21	Not noticeable	Bluish white	6
Blue Rose	12.07	5 mm. wide 10 mm. long	6 mm. wide 15 mm. long	22	Not noticeable	Bluish white	4 [2]
Calora	12.43	5 mm. wide 8 mm. long	6 mm. wide 13 mm. long	20	Not noticeable	Creamy	3
Edith	12.62	4½ mm. wide 10–11 mm. long	5½–6 mm. wide 15–16 mm. long	22	Some very small	Creamy white	7
Lady Wright	9.47	4 mm. wide 11 mm. long	6 mm. wide 15 mm. long	23	Not noticeable	Creamy white	5 [2]
Early Prolific	9.43	4 mm. wide 9–10 mm. long	6 mm. wide 13–14 mm. long	24	Some very small	Creamy	8

[1] The tests for starch were made by adding iodine to the cooking water.
[2] These varieties gave a test for dextrin.

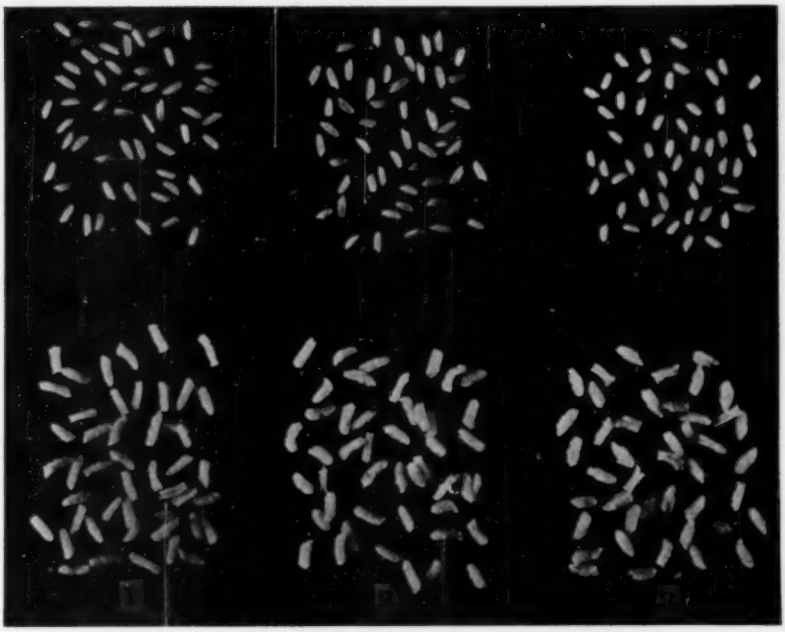

Fig. 1. Rice varieties: Upper series uncooked; lower series cooked. (1) Fortuna, (2) Edith, (3) Early Prolific.

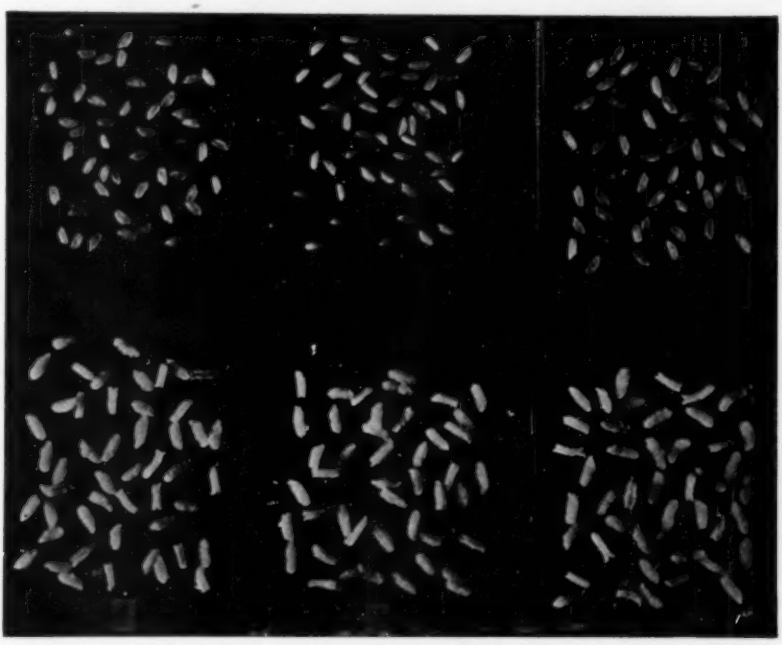

Fig. 2. Rice varieties: Upper series uncooked; lower series cooked. (4) Blue Rose, (5) Lady Wright, (6) Honduras.

water and produces a very soft and sticky rice. Figures 1, 2, and 3 illustrate the appearance of the rice before and after cooking.

In some of the varieties of rice, the centers of the grains were unusually hard, and boiling until these were softened tended to overcook the exteriors of the grains, causing stickiness or disintegration.

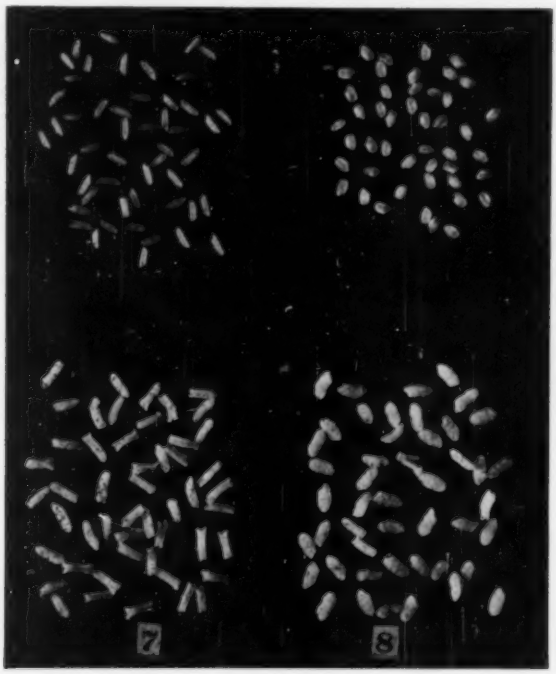

Fig. 3. Rice varieties: Upper series uncooked; lower series cooked. (7) Rexoro, (8) Calora.

After the rice samples were cooked to tenderness, they were drained, and washed by pouring cold water through to remove the soluble starch and separate the grains. The samples were then drained 5 minutes and weighed. Data with respect to the time of cooking, general appearance of the cooked rice (wholeness, stickiness, firmness), as well as the loss of starch in cooking water are shown in Tables I and II.

The percentages of absorption of water during the cooking period were determined by comparing the weights of the cooked samples with the weights of the original samples. The data for these values are recorded in Table III. While in every case there was some loss due to disintegration of the rice, these figures for absorption are comparable and the most accurate that could be obtained.

TABLE II

SCORES FOR THE DIFFERENT VARIETIES OF RICE

Variety	Shape of cooked grains	Wholeness of cooked grains	Stickiness of cooked grains	Firmness of cooked grains	Flavor of cooked grains
Rexoro	Slightly bent	Slightly broken	Slightly sticky	Holds shape; offers resistance yet tender	Slightly perceptible; neutral
Honduras	Slightly bent	Slightly broken	Slightly sticky	Holds shape; offers slight resistance	Perceptible; slightly desirable
Fortuna	Slightly bent	Slightly broken	Moderately sticky	Holds shape; offers slight resistance	Slightly perceptible; neutral
Blue Rose	Slightly bent	Slightly broken	Moderately sticky	Holds shape; offers slight resistance	Slightly perceptible; neutral
Calora	Straight	Slightly broken	Moderately sticky	Holds shape; offers slight resistance	Slightly perceptible; neutral
Edith	Slightly bent	Slightly broken	Moderately sticky	Holds shape; offers no resistance	Slightly perceptible; neutral
Lady Wright	Slightly bent	Moderately broken	Moderately sticky	Holds shape; offers no resistance	Slightly perceptible; neutral
Early Prolific	Slightly bent	Moderately broken	Moderately sticky	Partly holds shape; offers no resistance	Perceptible; neutral

In this connection, an attempt was made at the beginning of the work to measure absorption by difference calculated from the liquid evaporated during boiling. Evaporation per minute under standard conditions (without rice) was determined, but the figure obtained could not be used because of showing much more evaporation than actually took place. It was evident that the presence of rice in the water considerably reduced evaporation.

TABLE III

DETERMINATION OF WATER ABSORPTION

Variety	Weight of sample	Cooking time	Gain in weight	Water absorption $\frac{\text{Gain in weight of sample}}{\text{Weight of sample}} \times 100$
	Gms.	Min.	Gms.	P.ct.
Rexoro	80	16	172	215
Honduras	80	22	178	222
Fortuna	80	21	184	230
Blue Rose	80	22	168	210
Calora	80	20	155	194
Edith	80	22	161	212
Lady Wright	80	23	172	215
Early Prolific	80	24	180	225

The color of the cooked rice was judged by comparing the samples with each other.

Conclusions

It was concluded from this work that, since different varieties of rice require different cooking periods, rice should not be sold as mixtures of different varieties, as is now sometimes done; but it should be sold by variety name and by grade.

It was concluded, also, that the samples of rice tested should be ranked in order of desirability for cooking purposes on the basis of their scores in these tests (from most to least): (1) Rexoro, (2) Honduras, (3) Fortuna, (4) Blue Rose, (5) Calora, (6) Edith, (7) Lady Wright, and (8) Early Prolific. The less desirable varieties can be prepared with a minimum amount of breakage of kernels and a minimum degree of stickiness if the method of boiling described above for experimental lots is used.

THE SOFT WINTER WHEAT IMPROVEMENT PROGRAM FOR OHIO

E. G. Bayfield[1]

(Read at the Convention, May, 1931)

The production of soft red winter wheat plays an important part in the agriculture of Ohio. According to Foster,[2] Ohio produced during the period 1921 to 1925 an average of 33,218,000 bushels per year, or 14.9% of the total soft red winter production for the United States. The same writer also shows that a distinct upward trend in yield per acre occurred from 1866 to 1928.

Warren[3] recently reiterated his forecast that all commodities would reach pre-war prices during the next ten or more years. Wheat has already gone to extremely low price levels. Warren lists the need for higher yields per acre, more scientific research, and improving the quality of products produced among other methods for meeting the situation.

In 1929, members of the agricultural and milling industries in the three states of Ohio, Indiana, and Michigan commenced a cooperative program which promises to at least partially meet the present crisis in wheat production insofar as these three states are concerned. This

[1] National Milling Company Fellow for Ohio. Department of Agronomy (Cereal Chemistry), Ohio Agricultural Experiment Station, Wooster.

[2] Foster, L. G., 1930. Some factors affecting the movement of Ohio wheat. Ohio Agr. Expt. Sta. Bull. 458.

[3] Warren, F. G., 1931. Economic outlook for agriculture in the United States. Sci. Agr. 11: 467–486.

cooperative work is being carried on under the Tri-State Wheat Improvement Association of Toledo, Ohio. This Association aims to improve the quality of wheat by eliminating varieties of undesirable milling qualities, by reducing the number of varieties grown, and by means of a system of discounts to encourage the growth of certain varieties found to be the most desirable for the different areas throughout the three states.

Members of the milling industry recognized the need for more scientific research, and to further work along these lines the National Milling Company of Toledo established a fund to support a five-year fellowship in each of the three states. Each state was to undertake a specific phase or phases of the problem of improving the quality of the wheat grown in the Tri-State territory. The Ohio Experiment Station undertook the study of the effects of environment upon wheat quality. Such a study must necessarily continue over an extended period of years before reliable conclusions may be drawn.

At the present time the project has not progressed far enough to warrant publication of results. It is believed by the writer, however, that those interested in the various phases of the production and utilization of soft winter wheats, and cereal chemists generally, will be interested in the results coming from a research program due to the active cooperation of agricultural experiment stations and the milling industry. Published results in detail may be expected from time to time as circumstances warrant.

In 1929 approximately four hundred samples of wheat were collected as a preliminary survey from various farmers and experimental fields throughout the Tri-State area. These were shipped to the laboratories of the National Milling Company in Toledo, where all samples from each state underwent certain uniform analytical determinations. In the case of the wheat, these determinations consisted of weight per measured bushel, moisture, and crude protein. After milling, the moisture, ash, percentage absorption, and crude protein were determined on the flour. Finally, all samples were baked and subjected to a special fermentation test. The uniform handling of all material in a single laboratory provides many advantages which will be appreciated by all workers in the field who realize the difficulty of obtaining directly comparable results when different investigators, equipment, and technique are employed.

In the same year the first of the Tri-State "quality" plantings were made at thirty different locations. Nine varieties were included in this "quality" series of plots and the complete set of varieties was planted when circumstances permitted. The varieties consisted of Red Rock, Trumbull, Nabob, Berkeley Rock, Fulhio, Fultz, Michigan

Amber, Kharkov, and American Banner. Kharhov was included for the sake of comparison, although not a representative of the soft winter class of wheats; the other varieties were soft red winters with the exception of American Banner, a soft white winter wheat.

At the above locations a wide range of soils and climate is found. The major types of soils in the Tri-State area are represented and the locations were selected carefully, as soil and climate are two of the most important environmental factors which affect wheat quality. The Indiana plots were all located on experimental farms; while those located in Michigan and Ohio were at similar institutions, or were grown cooperatively by farmers who were supplied with seed and fertilizer, but who did not undertake the threshing of the samples used for the milling test.

In 1930 over five hundred samples were received from the three states for analysis and study. These samples consisted of those harvested from the special quality plots as well as other varieties, both old standard sorts and new hybrids. These latter, often unnamed varieties, are being examined carefully in order to prevent any new productions with poor milling qualities from getting into general distribution. A certain number of samples were also collected in Ohio for the purpose of studying the effect of varying fertilizer treatments applied either at the time of seeding or later in the life history of the plant.

From the environmental studies to date a few interesting trends are noticeable:

1. In the Tri-State area soil appears to have a more important influence upon the strength of the wheat produced than was ordinarily considered to be the case by earlier workers.

2. Climate will annually superimpose its influence upon the wheat grown on different soil types over the area concerned.

3. Fertilizer practices and crop sequence appear to influence the strength of the wheat grown to a considerable extent.

4. Weak varieties of soft winter wheats do not appear to be able to utilize as large an amount of available nitrates in the soil as do stronger varieties with the result that the ranking in strength of varieties tends to remain in the same order irrespective of their nitrate supply.

5. Correlation studies indicate that the baking test is the best measure of strength insofar as the different determinations employed are concerned.

Annual Report of Secretary-Treasurer

M. D. Mize

January 1, 1932

The same general form that was adopted last year has been retained, so that a direct comparison can be made between this report and the 1930 Annual Report. By making this comparison, you will notice that there is very little difference between the 1930 and 1931 receipts and expenditures, except in the case of The Experimental Baking Fellowship Fund and The Book of Methods Fund. Activities of the Book of Methods have been limited this year to cleaning up a few small expenses and collecting interest on the reserve fund. The entire cost of conducting the Baking Fellowship during 1931 has been incurred while the only revenue has been interest on the funds on hand. This leaves the fund practically depleted.

The assets of the Association Account have grown from $1,034.12 on December 31, 1929 to $1,865.81 on December 31, 1931. This makes a fairly safe size reserve fund which more than equals the entire expenses incurred during the whole year. By making the registration fee at our 1931 convention cover the cost of the programs and other incidental expenses by the Secretary-Treasurer's office in connection with the convention; the Receipts have exceeded the Expenditures made by the Association by $539.93 which is over $200.00 larger than last year's profit. Of course part of this increase was due to the large number of new members taken in during the year. This new method of making the Convention Registration Fee cover the entire cost of the Convention should by all means be continued. Even if only one-third as many new members had been taken in during the year, the Revenue would have exceed Disbursements by $423.93. The past year has seen many financial reverses and decreased revenues and salaries. Since this Association is not interested in making a large profit and accumulating capital, and since a reduction of $1.00 in the annual dues of the active members would have reduced the revenue of the Association Account by $414.00; your Secretary-Treasurer recommends that serious consideration be given at our 1932 convention of reducing the active membership dues to $6.00 unless the Association experiences a decrease in membership during the the first six months of the year.

DETAILED MEMBERSHIP STATEMENT DECEMBER 31, 1931

	Total	Active	Corp.	Hon.
Membership, Dec. 31, 1930	439	393	44	2
New members added during 1931	64	58	6	0
Members reinstated	2	2	0	0
	505	453	50	2
Members resigned and suspended for non-payment of dues during 1931	40	38	2	0
Members deceased	1	1	0	0
Members in good standing Dec. 31, 1931	464	414	48	2
	505	453	50	2

FINANCIAL STATEMENT

January 1 to December 31, 1931

RECEIPTS 1931			
Cereal Chemistry			
Membership dues			
Active	$1,099.50		
1931 Dues received in 1930	350.00		
Net 1931		$1,449.50	
Corporation	230.00		
1931 Dues received in 1930	250.00		
Net 1931		480.00	
Subscriptions, reprints, back numbers and advertising	3,084.90		
1931 Subscriptions received in 1930	495.00		
1931 Accounts receivable	189.13		
1932 Income receivable in 1931	*413.25*		
1930 Income received in 1931	*306.31*		
Net 1931		3,049.47	
Gift to Cereal Chemistry from Association Funds		200.00	
Interest on Invested Funds		135.25	
Miscellaneous Income		37.00	
Total Net Receipts 1931			$5,351.22
Association			
Membership dues (Active)	1,099.30		
1931 Dues received in 1930	350.00		
Net 1931		1,449.30	
Application Fees	171.00		
1931 Fees paid in 1930	3.00		
Net 1931		174.00	
Interest on Invested Funds		65.00	
From Registration Fees at Louisville Convention		138.01	
Total Net Receipts 1931			$1,826.31
Book of Methods Reserve Fund			69.89
Experimental Baking Fellowship Fund			
Interest on Invested Funds		55.71	55.71
TOTAL RECEIPTS OF ALL ACCOUNTS 1931			$7,303.13
DISBURSEMENTS 1931			
Cereal Chemistry			
Cost of editing, printing and mailing Cereal Chemistry and reprints	$4,331.18		
Net loss in Inter City Bank, Kansas City, Missouri, Closed on Dec. 29, 1926	332.26		
1931 Accounts Payable	651.71		
Petty Cash Fund in Washington, D. C., increased	*100.00*		
1930 Accounts paid in 1931	*18.00*		
Net Disbursements 1931		$5,197.15	
Profit 1931			$ 154.07

Association			
Expenses of President's, Vice-President's Office, News Letters	331.12		
Expenses of Sec'y-Treas.'s Office	362.75		
Committee Expenses	160.98		
Convention Expenses	162.90		
Gift to Cereal Chemistry	200.00		
Miscellaneous Expenses	68.63		
Net Disbursements 1931		1,286.38	
Profit 1931			539.93
Book of Methods Reserve Fund		23.60	
Profit 1931			46.29
Experimental Baking Fellowship Fund		2,416.96	
Net Disbursements for 1931			*$2,361.25*
TOTAL DISBURSEMENTS OF ALL ACCOUNTS		$8,924.09	

PROFIT AND LOSS ACCOUNT

Cereal Chemistry Assets 1930	$2,616.52	
Profit 1931	154.07	
Assets Dec. 31, 1931		$2,770.59
Association Assets 1930	1,325.88	
Profit 1931	539.93	
Assets Dec. 31, 1931		1,865.81
Book of Methods Reserve Fund 1930	329.69	
Profit 1931	46.29	
Assets Dec. 31, 1931		375.98
Convention Reserve Fund 1930	452.78	
Assets Dec. 31, 1931		452.78
Experimental Laboratory Baking Fund 1930	2,806.06	
Net Disbursements for 1931	*2,361.25*	
Assets Dec. 31, 1931		444.81
Total Assets December 31, 1931		$5,909.97

ASSETS AS OF DECEMBER 31, 1931

U. S. National Bank—Checking Account	$ 186.69
Cash on Hand	473.93
U. S. National Bank—Savings Dept.	254.05
First National Bank—Savings Dept.	671.13
Petty Cash Fund in Washington, D. C.	200.00
Building & Loan Stock in Kansas City	2,000.00
Building & Loan Stock in Omaha	1,500.00
Building & Loan Stock in Minneapolis	1,500.00
1931 Accounts Receivable	189.13
GROSS ASSETS	$6,974.93
LIABILITIES	
1931 Accounts Payable	*651.71*
1932 Income Receivable in 1931	*413.25*
NET ASSETS	$5,909.97

Note: All amounts in italics are negative amounts and are subtracted from the other amounts in the same column.

BOOK REVIEWS

The Law of Patents for Chemists. By Joseph Rossman, Patent Examiner of Chemical Inventions, U. S. Patent Office. Published by The Inventors Publishing Co., 1266 New Hampshire Ave., Washington, D. C., 1932. 304 pp., 6 x 8½; Cloth. Price, $3.50.

In an expert manner and in non-technical terms Mr. Rossman devotes the whole of his book to patent law in its relation to the chemist inventor. Although expressly written for the chemist, more than half of its contents is of equal value to the non-chemist inventor who has had limited or no experience in patent matters.

The introduction to the main part of the book is assumed necessary because graduate chemists and chemical engineers enter upon their professional duties with an almost complete ignorance of patent law and what constitutes patent rights. The author proceeds from a discussion on the value of patent law knowledge to a chemist to the subject of chemists as potential inventors and the value of patents to them. We learn that patents may be used offensively and defensively depending upon whether they are to establish a monopoly, to prevent others from obtaining a monopoly, or to serve as pawns in obtaining concessions from holders of other patents. Seeking patent rights for either purpose is preferable to trying to keep an invention secret. Secrecy offers no protection against inventions by other investigators. Patent office records show that about 4% of all patent applications overlap or interfere thus indicating that independent investigators make similar inventions. The one seeking patent rights gets the recognition if he proves an invention. Further preliminary instruction of reader consists of discussion on when to apply for a patent, what constitutes publication of results for scientific priority incidental to patent rights, and what is a chemical patent. The author classifies them as being process, composition, form, machine, or improvement patents.

Regardless of type each patent involves something more than a discovery. There must be an invention; an element of newness; a factor previously unknown. Complete chemical patent applications are exhibited to illustrate the various types, which are subsequently discussed in more detail. To be entitled to a patent, an inventor must exercise his creative faculties to produce something that is new and is useful; he must have been diligent in perfecting his invention; he must make full disclosure of his invention which in turn must be definable by statute; and he must file application before he is debarred by law.

With this introduction and setting forth of essential principles in patent law as a background the chemist reader is given definite and non-technical instructions regarding procedure in preparing and making application for patent rights on an invention. Particularly enlightening is the chapter devoted to preparing and stating the claims pertaining to the invention. It is significant to learn that a process claim gives no protection for the resulting product and vice versa. Also that in chemical inventions product claims are the most desirable form of patent application with process claims being preferable to apparatus claims for carrying out process. Numerous examples of specimen claims are given from completed patents. Chapters on "Prosecution of the Patent Application," "Interferences," and "Correcting the Patent" complete the section of the book devoted to obtaining a patent.

Recapitulating under the title of "Rights Under Patents" Mr. Rossman expands on the essentials of a valid patent, on patent rights, and on enforcement of patent rights in which he lists 28 defenses in suits for infringement.

The book is made complete by a final section containing general and miscellaneous information and an 18-page glossary of legal patent terms, and appendixes including an illustrative complete chemical patent, a selected bibliography of books and periodical articles on patents and patent law, and a table of patent cases.

E. S. STATELER.

The Structure and Composition of Foods, Volume I, Cereals, Nuts, Oil Seeds. By Andrew L. Winton, Ph.D., and Kate Barber Winton, Ph.D. Published by John Wiley & Sons, New York. 1932. Price $8.50.

This book is a very remarkable and comprehensive addition in the field of food chemistry and food microscopy. The handling of the subject matter is simplified and direct. The authors have a very logical method of classification taking the subjects by economic groups, by parts (fruits, seeds, leaves), by families, by genera, and by species. Each subject is also treated according to macroscopic structure, microscopic structure, and chemical composition. Where possible they also give microscopic and chemical composition of the separated parts as found in manufactured products or as often separated in the laboratory for study.

One commendable feature of the work is that they do not repeat great amounts of data available in most text books, such as the preparation of solutions and reagents. They do include excellent lists of amino acids, fatty acids, carbohydrates, and glucosides, that are very pertinent to the subject matter.

One special chapter deals with starch, its structure and composition; and the various commercial starches. Plates are given showing the microscopic details and structure of the different starches. The list is very complete and decidedly valuable. The chapter on oils is not so comprehensive, and there is no chapter on proteins which may have been an interesting addition even though a highly controversial subject. In all, thirty-six different commercial starches are described.

To give an excellent idea of the thoroughness of the authors, one need only give the topics treated in Part I, dealing with Cereals. These subjects include the Seeds of the Ginkgo Family, the Grass Family, the Buckwheat Family, the Goosefoot Family, the Amaranth Family, the Pink Family, the Water-Lily Family, the Pea Family, and the Nuts of the Oak, Horse-Chestnut and Water Chestnut Families. Each division being very complete, there being forty-four genera and species under the family Gramineae; The Cereal and Weed Seeds of the Grass Family.

Throughout the volume there are many tables giving chemical composition or scientific data relative to the various subjects. These are very important not only in giving a better understanding of the subjects under discussion, but the authors have gone further and included tables showing the chemical composition of many of the chemical constituents as usually reported. For instance, they not only show the total amount of globulins in peanuts, but give you their composition and the basic amino acids of these globulins.

Part II takes up specifically the Oil Seeds and includes the Oil Nuts. The general scheme of handling the subjects and presenting the subject matter is carried out with the same detail and faithfulness as in Part I. In this section more attention is paid to those chemical constituents of more importance in oily matter, such as amino and fatty acids and specific gravity, melting point, saponification number, iodine number, etc., of the various oils of the subjects discussed. To the food chemist this part may prove the most interesting of the three.

Part III deals with the Forage Plants, and naturally, of necessity is limited in comparison with the other products, but the discussion is excellent and the list quite extensive. Even here the usual procedure is carried out giving the macroscopic and microscopic structure along with the chemical composition. This part, while being of more importance to feeding of animals has a proper place in any work on food chemistry.

In spite of all the valuable data and information on the chemical composition of these food products, the macroscopic and microscopic features of the book will probably prove the most interesting. These phases show the most originality and are perhaps more representative of the personal work of the authors. The 274 illustrations are made entirely from drawings by the authors themselves and show a thoroughness and technique beyond compare.

In the macroscopic sections they expertly and adequately describe the entire botanical features of the fruit parts of the subjects. These descriptions are so complete that even the beginning student should have no trouble in determining species of the same family. Also many of the drawings make the macroscopic characteristics of the subject more apparent and are a great aid.

The microscopic features of the work, including drawings and descriptions have a decided clarity that should enable anyone acquainted with microscopic work to readily identify like subjects. These drawings and descriptions include all parts of the subject such as spermoderm, perisperm, endosperm, epiderm, and other layers, and show the arrangement and structure of both tissue and cell construction. It is

impossible to adequately describe these drawings, but their technique and completeness show years of association with the work.

We would say that the work is primarily intended for advanced study and not for beinning students. To the graduate, and particularly the industrial scientist, it is a much needed work of reference and approaches the cyclopedia.

S. J. LAWELLIN.

BULLETIN REVIEW

Report of Operation, State Testing Mill, Crop Season of 1930. Bulletin 7, Minnesota State Department of Agriculture, Dairy and Food, St. Paul, February, 1932. 50 pp. By H. A. Halvorson, Director.

A review is given of the accomplishments of the State Testing Mill for the periods 1921 to 1926, accompanied by a general discussion of the mill's equipment, method of operation, and objectives. It is explained that, while extensive studies were undertaken for the crop years 1927, 1928, and 1929, similar to those reported for 1921 to 1926, no regular bulletins were issued largely on account of financial retrenchments. Reference is made to a number of papers in the literature which record investigations consummated at the mill.

A general discussion is given of the characteristics of the 1930 Minnesota wheat crop, and complete data with respect to large scale milling and baking tests are presented for 40 representative lots of Minnesota-grown wheat. For comparative purposes, 12 lots of wheat grown in the Dakotas were also tested. The relative merit of the 1930 crop as compared with the wheat of the 1921 to 1926 crops is discussed in some detail. In addition to the usual routine tests for moisture, protein, and ash in the flour, special tests were made with respect to the pH of the flour, as well as its diastatic activity. Data are also recorded comparing the moisture content of different mill streams. A study was also made correlating the ash content of certain mill streams with the ash content of the original wheat. Mention is made of special tests conducted to determine the milling and baking properties of Marquillo wheat. Certain comparisons are also given with respect to analyses of the feeds produced in the Testing Mill and commercial mills.

D. A. COLEMAN.

ERRATUM

TESTING WHEAT VARIETIES FOR MILLING AND BAKING QUALITY

C. O. SWANSON AND E. H. KROEKER

Vol. I, No. 1, p. 22, 2d paragraph, 5th line, "The reading is from left to right," should read "The reading is from right to left."